

Pulp and Paper Industry: Microbiological Issues in Papermaking

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Preface

Increased system closure and reuse of treated effluent, together with a greater use of recycled fiber derived from comingled sources, are all factors that lead to increased microbiological activity within the mill. This leads to a number of well-reported impacts including visible “biofilms/slimes,” odor within the mill and the finished product, and unwanted “microbial bioburden” within the final paper sheet, especially in heavier packaging grades. As a consequence of the dominant species in the system, different runnability and production problems give rise, which is a function of the mill conditions. To control these problems, papermakers consider important not only to control the microbiological activity but also to prevent their growth. Furthermore, the traditional use of biocides has been modified to carry out the actual environmental considerations. To know the actual situation of the paper industry, this book presents a review of the microorganism sources, the consequences of the microbiological activity upon the actual systems of paper and board manufacturing, and the current state of the different alternatives for its prevention, treatment, and control considering the impact of the actual technological changes in papermaking on the control programs. Finally, the book presents the trends regarding the future of the microbiological control in papermaking systems.

Glossary

Aerobic bacteria These bacteria require oxygen for respiration.

Algae Algae are simple celled plants and (like all plants) contain chlorophyll. This traps energy from the sun and uses that energy to convert nutrients and carbon dioxide (which are dissolved in the water) into growth.

Alpha-amylase Amylase that catalyzes the hydrolysis of internal alpha-1,4-bonds in starch molecules and starch breakdown products.

Amylase An enzyme that catalyzes the breakdown (hydrolysis) of starch. Names such as alpha amylase or endoamylase, beta-amylase, amyloglucosidase (glucoamylase), etc., refer to enzymes that attack starch or starch break down products in slightly different ways.

Anaerobic bacteria These bacteria do not require oxygen for respiration.

ATP Adenosine triphosphate, an energy-rich molecule that is important as a source of energy in cells.

Bacteria Unicellular, prokaryotic, microscopic, generally heterotrophic organism present in great numbers in soil and in water; largely responsible for decomposition of primary and secondary produced organic matter and for mineralization of its constituent elements, C, N, P, S etc. The bacteria are one of the three domains of life, the other being Archaea and Eukarya (eukaryotes).

Bacteriophage A virus that infects bacteria and multiplies within the cells. Usually, a bacteriophage can only infect a limited range of bacterial strains.

Bacteriostatic Chemical compound that inhibit the growth of bacteria.

Biocide Chemical compounds that kill microorganisms. Bactericides, fungicides, etc., are examples. In the paper industry, they are typically used to control slime. These are of two types: oxidizing and nonoxidizing biocides.

Biorrosion Corrosion processes initiated by or accelerated by the growth of microorganisms at the metal surface.

Biodegradation The act of degrading a molecule to one or more smaller molecules by biochemical mechanisms (e.g., enzyme action).

Biodeispersants Chemical compounds that act as “biopenetrators,” opening the biofilms and allowing the biocides to penetrate the layer of the slime. They can also prevent the formation of nonbiological deposits, which could be a nutrient source for the microorganisms, facilitating the action of biocides.

Biofilm A slime-like matrix composed of extracellular polymeric substances within which a consortium of microorganisms flourishes. These biofilms may either grow over surfaces, or occupy voids in a porous medium.

Biofouling Any deleterious event in which a definable biological activity causes a deterioration in and engineered or natural process or system. Deleterious effects range from clogging, corrosion, and plugging to gas production and bioaccumulation.

Biological oxygen demand (BOD) A measure of the amount of oxygen consumed in biological processes that break down organic matter in water. The greater the BOD, the higher the degree of pollution.

Bioluminescence The production of light by living organisms.

Biosensor A device, especially an electrochemical device, that detects some biological event (for example, respiration, enzymic activity, binding to an antibody) and converts it into an electrical signal that it reports quantitatively and in real time.

- Cellulases** A family of enzymes that hydrolyze β -1, 4-glucosidic bonds in native cellulose and derived substrates.
- Chemical pulp** Fibrous material obtained by removal from the raw material of a considerable part of those noncellulosic compounds that can be removed by chemical treatment (cooking, delignification, bleaching).
- Clogging** The generation of a mass that interferes with physical functioning of a porous medium. Clogging can be formed through the maturation of biofilms fouling the media and may become complex in structure.
- Closed-cycle** A mill or industrial plant that has little or no process effluent.
- Chemical oxygen demand (COD)** A measure of the oxygen required to oxidize all compounds in water, both organic and inorganic. COD is more widely used because it is a simple procedure and includes the effects of nonbiodegradable organic matter, which can account for up to half of the material discharged.
- Colony forming units (cfu)** When microorganisms do grow on agar media, they commonly form visible distinguishable structures composed mainly of cellular material, which are called colonies. Each of these colonies is considered to have formed from a single colony forming unit that may be a single cell or a clump of cells. By appropriate mathematical relationships of the dilution of the sample and the area of the agar inoculated, it is possible to predict a population as either cfu/mL (for liquids), cfu/g (for solids), or cfu/cm² (for surfaces).
- Culture** The act of successfully growing a unique strain or a consortium of microorganisms; (noun) a viable collection of a single strain of microorganisms that has been selectively grown in vitro under controlled (laboratory) conditions.
- Dispersant** These are a class of surfactant chemical used in papermaking systems to reduce deposits of pitch and slime, in deinking systems to disperse the ink particles, and in coating formulations to keep the clay particles in suspension.
- Dissolved and colloidal substances** Usually derived from wood and usually having a negative charge, tending to interfere with retention aids and other papermaking additives.
- Enzyme** A protein that has the ability to direct or catalyze a chemical reaction.
- Extracellular polymers (EPS)** The polysaccharide material produced by microorganisms that surround the microbial cells, which enhances the attachment to surfaces.
- Fungi** A kingdom of life forms that are eukaryotic, mycelial or yeast-like, heterotrophic, lacking in chlorophyll, sexually and/or asexually reproductive, and mostly aerobic.
- Glycocalyx** A general term referring to extracellular polymeric material produced by some bacteria composed of carbohydrates, lipids, and proteins.
- Iron oxidizing bacteria** These bacteria are able to oxidize iron by any means from a reduced form of iron (ferrous form) to an oxidized (ferrous) state.
- Iron-reducing bacteria** These bacteria are able to reduce iron by any means from an oxidized form (ferric) to a reduced (ferrous) state.
- Iron-related bacteria** All of those bacteria that are able to accumulate iron in another form beyond that for basic metabolic functioning. These accumulated iron compounds generally collect within the slime (EPS) around the cells and gradually harden (crystallize) over time.
- Limiting nutrient** A major nutrient that is in short supply and restricts the growth of a biomass. Limitations could also be created by the limiting nutrient distorting the ratios of nutritional elements outside of range that would support growth.
- Macrofouling** An intense and/or widespread form of biofouling.
- Mechanical pulping** Mechanical pulping uses revolving disks to grind wood chips into pulp. Water is added to the process to reduce wood damage resulting from heat and friction. One of the nonfibrous elements that is not removed during mechanical pulping is lignin, an organic material that binds fibers of cellulose together in the wood. It is the presence of lignin that is primarily responsible for low durability and yellowing with age. Mechanical (or groundwood) pulp is inexpensive to produce and generates the highest yield.
- Microbial induced corrosion** Corrosion processes initiated by or accelerated by the growth of microorganisms at the metal surface.
- Microorganism** An organism of microscopic size, including bacteria, fungi, and viruses.
- Non-oxidizing biocide** A non-oxidizing biocide is one that functions by mechanisms other than oxidation, including interference with cell metabolism and structure.

Oxidizing biocides Agents capable of oxidizing organic matter (e.g., cell material, enzymes, or proteins that are associated with microbiological populations resulting in death of the microorganisms). The most commonly used oxidizing biocides are based on chlorine or bromine (halogens) that liberate hypochlorous or hypobromous acids on hydrolysis in water. The exception is chlorine dioxide, a gas that does not hydrolyze but that functions in the same way.

Pectin A highly hydrophilic polysaccharide built up of monomers of an important component of cell walls.

Pectinase Pectinase also known as polygalacturonase is the collective term for a row of enzymes that are able to break down or to transform pectins.

Polysaccharides Carbohydrates that hydrolyze to yield more than 10 molecules of a monosaccharide (cellulose and starch are glucose polymers).

Planktonic organisms Free-living organisms (that swim or float in the water phase).

Plate count Test method for the determination of a microbiological contamination and/or for testing the efficiency of biocides. It is based on the principle that one cell grows out to form one colony. After incubation of a certain amount of the sample, by the use of a certain culture media and for a certain period, the number of colonies formed is counted.

Semimechanical pulping As the name implies, it is a two-stage process that uses a chemical mixture (most commonly sodium sulfite and alkaline salts) to soften lignin, followed by a disk refiner to fiberize the cooked chips. However, a substantial portion of the lignin still remains, and pulp yield (60%–80% of the original wood) is less than that of mechanical pulping. Semimechanical pulping produces stiff fibers, and is generally used for corrugated board, roll cores, and containers. Semimechanical pulp is not used for paper intended for writing or printing.

Sessile organisms Organisms that are attached to surfaces.

Slime control Inhibition of slime formation.

Slime deposits Deposits in the papermaking system characterized by some degree of microorganism activity, but also consisting of various combinations of organic and inorganic material.

Slimicide Chemical product used to inhibit the formation of slime.

Sloughing The act of a slime, for whatever reasons, breaking up and releasing particles (from the slime) to the water passing over the slime.

Sulfate-reducing bacteria Strict anaerobes that oxidize organic substrates and use sulfate or other oxidized sulfur compounds as terminal electron acceptors. The reduced sulfur is released into the environment as hydrogen sulfide gas. They produce a very bad smell; a black iron sulfide precipitate is formed; and they are actively involved in microbiologically induced corrosion and form explosive gas at certain concentrations.

Total viable counts The total number of culturable bacteria (per volume or area) in a given sample.

Vegetative bacteria Bacteria that are devoid of spores and usually can be readily inactivated by many types of germicides.

Yeast Yeasts are a group of unicellular fungi which exist almost everywhere in nature. Commonly used to leaven bread and ferment alcoholic beverages.

Abbreviations

AET	Alliance for Environmental Technology
AKD	Alkyl ketene dimmer
ASA	Alkenyl succinic anhydride
ATP	Adenosine triphosphate
BCDMH	1-bromo-3-chloro-5,5-dimethylhydantoin
BHAP	2-bromo-4-hydroxyacetophenone
BIT	1,2-benzisothiazolin-3-one
BrMEH	Bromine methylethylhydantoin
cfu	Colony forming units
CLSM	Confocal laser scanning microscope
DBMH	1,3-dibromo-5,5-dimethylhydantoin
DBNPA	2,2-dibromo-3-nitrilopropiamide
DBNPA	2,2-dibromo-3-nitrilopropionamide
DCS	Dissolved and colloidal materials
DCDMH	1,3-dichloro-5,5-dimethylhydantoin
DLVO	Derjaguin-Landau-Verwey-Overbeek
DNA	Deoxyribonucleic acid
DOT	Department of Transportation
ECAS	Electrochemically activated solutions
EOW	Electrolyzed oxidizing water
ECAW	Electrochemically activated water
EPA	Environmental Protection Agency
FISH	Fluorescence in situ hybridization
FRO	Free residual oxidant
GMP	Good manufacturing practice
GRAS	Generally recognized as safe
HACCP	Hazard Analysis Critical Control Points
MBT	Methylene bithiocyanate
MCA	Monochloramine
MIC	Minimum inhibitory concentrations
MIT	2-methyl-4-isothiazolin-3-one

MSC	Minimal sporicidal concentration
NOECs	No observable effect concentrations
OIT	2-n-octyl-4-isothiazolin-3-one
PAA	Peroxyacetic acid
PCC	Precipitated calcium carbonate
PCR	Polymerase chain reaction
PiBa	Pigmented Biofilm-Forming Bacteria assay
ROS	Reactive oxygen species
RTU	Relative toxicity units
AgNPs	Silver nanoparticles
TCMTB	2-(thiocyanomethylthio)benzothiazole
THPS	Tetrakis (hydroxymethyl) phosphonium sulfate
TOX	Total organic halogens
TOC	Total organic carbon

General Introduction

1.1 Global Pulp and Paper Industry

The pulp and paper industry is one of the largest industrial sectors in the world. It is also an important source of employment in many countries. A sustainably managed pulp and paper industry can bring many benefits to the local economy and people, particularly in rural areas. Pulp and paper is produced in every part of the world. The largest producer countries, United States, China, Japan, and Canada, make up more than half of the world's paper production, which is 400 million tons a year. Around the world we use more than 1 million tons of paper every day. Our paper consumption is escalating, particularly in emerging markets such as China. Pulp and paper is primarily made from wood fibers originating from natural forests or pulpwood plantations. Recycled fiber and other fiber sources such as agricultural residue are also used, and recycled fiber is becoming more commonly used in pulp and papermaking. Many global pulp and paper companies are moving their production to southern regions because lower production costs and proximity to fast-growing pulpwood plantations. Hence, responsible pulpwood plantations practices are urgently required.

Pulp and paper mills are big business around the world, generating \$563.6 billion in revenue during 2013. Over the past 5 years, revenue from the global pulp and paper industry is expected to increase at an average annual rate of 0.4%, driven by strong performance in paperboard packaging and tissue products. During the global economic downturn in 2009, however, industry revenue dropped a sharp 19.2% because of a severe drop in pricing and shipment volumes. Demand for printing and writing paper dropped from a structural change toward digital media and lower advertising spending from the global recession, particularly in mature markets in Europe and North America. Paper mills are generally large and contain sizable and heavy machinery used in the production of pulp, paper, and paperboard. The mills are large because small-scale production is too costly on a per-output basis. The sort of machinery required for large-scale production is expensive and therefore capital expenditure in the industry is substantial. That is the main reason for a high level of capital intensity; an estimated \$0.86 of capital is required for every \$1.00 spent on labor. The industry's capital intensity has increased slightly since 2008, mainly from increased capital investment in more efficient manufacturing machinery with lower energy requirements.

1.2 Microbial Problems in the Paper Industry: General Aspects

The pulp, paper, and board industry is continuously seeking to improve its economic and environmental performance (Bajpai, 2010). The efficient use of virgin and recycled wood

fibers, efficient water reuse, and control of emissions to environmental media are some of the key factors in this respect. In these contexts, the awareness of microbiological issues can be crucial to production performance. Table 1.1 shows industry changes resulting in increased microbial problems (Gudlauskis, 1996; Mattila-Sandholm and Wirtanen, 1992; Klahre et al., 1997; Dexter, 1996; Cantrell, and Lefevre, 1989; Robertson, 1995). The most significant and recent papermaking trends include (Rice, 2001):

- Conversion from acidic to neutral/alkaline papermaking
- Increased use of chemical additives
- Increased use of recycled fiber
- Closure of water systems

There are several benefits of conversion from acidic to neutral/alkaline papermaking. These include improved stability on aging, reduced costs, higher strength properties, reduction in energy consumption, decreased corrosion, increased productivity, and less complex systems. The most important aspect of this conversion is the replacement of alum and clay and titanium dioxide fillers with calcium carbonate. In addition, new retention aids, sizing and wet strength resins have become essential. Problems reported include reduced drainage and dryer efficiency and sheet quality, with an increase of holes, web breaks and pitch deposition problems. As the pH shifted toward the 7.0–8.0 range, the microbial populations shifted, and the population of freshwater microorganisms increased. Consequently, many of the common slime-control programs became less cost-effective or, in some cases were not effective at all.

New chemical strategies have become important for effective and well-organized management of the wet-end process for meeting the requirements of improved paper quality and increased productivity, taking into consideration the recent papermaking trends. Several types of chemical products are being used for sizing, retention, formation, drainage, distribution of fines, and microbiological and deposit control. Papermaking chemicals, namely processing chemicals and functional additives, account for more than 20% of world pulp and paper producers' total raw material expenditures.

Table 1.1: Industry changes resulting in increased microbial problems

Conversion from acidic to neutral/alkaline conditions
High-speed machines
Closure of water system
Increased use of recycled pulp
Increased use of fillers
Increased use of chemical additives
Lighter weight grades
Boilout interval changes

Based on Robertson (2009).

Recycled fiber is an inexpensive source of fiber. Today, more than half of the global amount of fibers used in papermaking is recycled fibers. However, recycled fibers consist of increased amounts of short fiber and is often contaminated with deinking chemicals, glue, and other substances. This results in increased deposit problems, such as slime and stickies. The contaminants can also lead to an increased consumption of chemical additives. The use of retention aids is also required to maintain the stable paper quality.

The amount of water required to make 1 ton of paper has decreased significantly over the past 20 years. The benefits of water systems closure include reduced treatment costs; increased system temperature; lower losses of fibers, fillers, and chemicals; and more stable operating conditions. Increased whitewater closure changes the environment and growth conditions for microorganisms in the papermaking process. The changes such as lower dissolved oxygen content, longer retention time, increased temperature, and increased total solids create conditions for microbial growth which result in product quality, runnability, and safety issues. Microbiological growth can result as either slime deposition, formation of volatile gases, or spoilage-producing acids that affect the bonding of organic compounds, which lead to material degradation, such as additives and wood fibers. The buildup of higher concentrations of dissolved and colloidal materials within the system, leads to pitch problems and increase of suspended solids, buildup of thermal energy, microbial activity, and corrosion. These problems result in poor utilization or an increased consumption of chemical additives. The paper quality and the performance of machine are also affected.

These process modifications may make microbiological problems worse and decrease mill performance. Running the process under closed conditions leads to accumulation of nutrients and higher oxygen demand, which in turn rapidly results in anaerobic conditions. This favors fermenting organisms that form low-chain fatty acids, causing malodors (Bennett, 1985). Maukonen et al. (2006) observed *Desulfovibrionales*-related bacteria in paper mill environments. Under such conditions, microbially influenced corrosion is also favored and this can affect electrical control equipment causing serious operational problems (Blanco et al., 1996).

Microbial problems in papermaking have been understood for decades (Beckwith, 1931; Appling, 1955), and the expansion of production plants has provided more space and opportunities for microbial growth. Paper consists of a web of pulp fibers derived from wood or other plants from which lignin and other noncellulose components are separated by cooking with chemicals at a high temperature (Smook, 1992). Before pulp is made into paper, it undergoes several steps called stock preparation. This is conducted to convert raw stock into finished stock which is known as furnish for the paper machine. The pulp is prepared for the paper machine including the blending of different pulps, dilution, and the addition of chemicals (Biermann, 1996). The raw stocks used are the various types of chemical pulp, mechanical pulp, and recovered paper and their mixtures. The quality of the finished stock essentially determines the properties of the paper produced. Raw stock is available in the form of bales,

loose material, or, in the case of integrated mills, as suspensions. Stock preparation consists of several process steps that are adapted to one another as fiber disintegration, cleaning, fiber modification, and storage and mixing. These systems differ considerably depending on the raw stock used and on the quality of furnish required. For instance, in the case of pulp being pumped directly from the pulp mill, the slushing and deflaking stages are omitted. The operations practiced in the paper mills are: dispersion, beating/refining, metering, and blending of fiber and additives. After stock preparation, the slurry is formed into the desired type of paper at the wet end of the paper machine. The basic elements of the paper machine are: headbox, wire section, press section, dryer section, and reel. The actual design of these elements mainly depends of the type of paper being produced. When the slurry has reached the paper machine, it first enters the headbox where the mixture is evenly spread over the entire wide of the paper machine. Consistency of the stock flow entering the paper machine headbox is typically 2–10 g fiber per kg water (Biermann, 1996; Smook, 1992). The web consistency increases to 15–25% after drainage on the wire or forming section. Mechanical compression removes water on the press section. The web consistency increases to 33–55% depending on the paper grade and press section design. After the press section, the web enters the dryer section where the remaining water is removed by evaporation. A small amount of moisture (5–9%) remains in the paper even after the dryer section (Kuhasalo et al., 2000). The water leaves the paper mill also as wastewater to the wastewater treatment plant. This means that even in the most closed plants, fresh water is needed to compensate this loss of water. The water is reused and cycled in several circulations in the paper mill. The water removed in the wire part of the paper machine is discharged into the wire pit and is used to dilute the stock fed to the paper machine. The diluted stock is pumped to the headbox and ahead to the wire section. This system is called short circulation (Weise et al., 2000). A part of the water removed in the wire and press section that is not led back into the headbox is used in the early stages of the papermaking. These waters compose a long circulation of the paper machine and are used to adjust the consistency and to improve the material and heat economy (Ryti, 1983). This water, which has been used at least once before, is called whitewater. It is also processed using several means. Modern paper machines can operate with very low fresh water consumptions with an optimized water usage. Usually the degree of closure varies between 2 and 20 m³/ton of produced paper depending on process and water processing technologies used (Weise et al., 2000; Bajpai and Bajpai, 1999). This leads to accumulation of various substances into water cycles. The dissolved and colloidal fractions are particularly difficult to separate (Wearing et al., 1985; Kokko et al., 2004). In closed loop mills where whitewater is recycled for dilution of the pulp, microbial contamination is further aggravated. Actually, closing up of whitewater system contributes to the cycling of nutrients and constant recontamination of the process. Factors such as pH, temperature, and the levels of organic nutrients also play a significant role in slime development. The conditions normally found in the paper machine are pH 5–8, 20–78 °C, and an abundance of nutrients are excellent environment for the growth of bacteria and fungi. The additives—starch, glues, and coatings used in the

mill—are excellent food sources for most of the microorganisms. Nutrients from pulp and whitewater are coated on surfaces to produce films of concentrated food that become the basis for microbiological growth and activity. Changes in equipment design, use of complex chemical mixtures of additives, changes in operating practices from high to low grammage grades, bad housekeeping, and storage of pulps, recycled fiber, and sludges are the major factors that aggravate the slime problem. Surface water supply from lakes, rivers, ponds, and wells can also be a serious source of inorganic nutrients (example iron, sulfur) and bacterial contamination.

The changes in papermaking have resulted in an increasing number of problems resulting from slime deposits caused by an increase in microbiological activity (Blanco et al., 1997). Slime is the generic name for deposits of microbial origin within the paper process. Slime is defined as the accumulation of microbial cells immobilized and embedded within an organic polymer matrix of exopolysaccharides (EPS), mixed in different proportions with fibers, fines, fillers, and other materials present within the paper process. By definition, EPS are located at or outside the cell surface. Their composition may be controlled by different processes, such as active secretion, shedding of cell surface material, cell lysis, and adsorption from the environment (Wingender et al., 1999; Gessey, 1982; Sutherland, 1990, 1994, 1995, 1998, 1999a,b,c, 2001). Some of the functions of the EPS matrix are adhesion to surfaces, aggregation of bacterial cells in flocs and biofilms, stabilization of the biofilm structure, formation of a protective barrier that provides resistance to biocides or other harmful effects, retention of water, sorption of exogenous organic compounds for the accumulation of nutrients from the environment, and accumulation of enzymatic activities, such as digestion of exogenous macromolecules for nutrient acquisition. A modern concept is that EPS allow microorganisms to live continuously at high-cell densities in stable mixed population communities. In other words, the EPS matrix is a medium allowing cooperation and communication among cells in microbial aggregates. Stable, close proximity of the bacteria requires that the cells be held together by the EPS (Wingender et al., 1999).

Increased use of recycled fibers causes constant contamination with bacteria (Blanco et al., 1997). The conditions normally found in paper mills are conducive for microbial growth. The critical areas of the process are the wet-end, coating section, and the size emulsion. At the wet-end of the machine slimes are usually found underneath the wire frame, on the surface of the foils, the suction boxes, the whitewater tanks, and the clarifiers. Recycling, as part of the paper cycle, plays an important role in the sustainable development of the paper industry (Bajpai, 2013). A direct consequence of moving toward higher recycling rates is the change to more heterogeneous paper sources. This leads to a lower recovered paper quality or, in other words, to more contaminated raw materials, from the point of view of organic, inorganic and microbial content. For example, recycled fibers can contain as many as 1000 times more microorganisms than virgin fibers from storage and transport conditions of the recycled fibers (Verhoef et al., 2002; Verhoef, 2005).

A paper mill provides a favorable environment for the growth of microorganisms (Klahre et al., 1996, 1997; Hassler et al., 2007; Robertson, 2009). Microorganisms enter in uncontrolled quantities with the process water, materials, and additives and also through the air. Water is available, biologically degradable material is abundant, and the range of operating temperatures is favorable for mesotrophic organisms which encounter a variety of habitats from fully aerobic to fully anaerobic, thus allowing for high biodiversity (Lahtinen et al., 2006). Under these conditions, excessive microbial growth results.

The level of hygiene in the paper and board industry is also very important because the end-products are often in contact with foodstuffs. The microorganisms are mainly bacilli, enterobacteria, pseudomonads, or actinomycetes, but yeasts, molds, anaerobic sulfate-reducing bacteria, and clostridia may also be found (Harju-Jeanty and Vaatanen, 1984; Raaska et al., 2002; Suihko and Hoekstra, 1999). The growth of clostridia, coliforms, *Bacillus cereus*, and staphylococci in the papermaking process is harmful to product hygiene (Pirttijarvi et al., 1996; Sorrelle and Eelgard, 1992). Aerobic and anaerobic spore-forming bacteria, such as bacilli and clostridia, are not destroyed during the drying stage of papermaking. From the safety point of view, these are the most important microorganisms (Hughes-van Kregten, 1988; Pirttijarvi, 2000; Robichaud, 1991; Väisänen et al., 1989). The machinery slime can also contain polymers of microbial origin, fibers, and inorganic precipitates. Common bacteria detected and identified from paper-machine slimes include enterobacteria, bacilli, pseudomonads, and *Clavibacter* spp. The total number of microbes in the slime can reach 10^{12} colony-forming units/mL. Few pathogens, such as *B. cereus*, can also be found in these paper-machine slimes. Anaerobic bacteria, such as sulfate-reducing bacteria, can be involved in the initiation and progress of corrosion (Bennett, 1985; Harju-Jeanty and Vaatanen, 1984; Väisänen et al., 1994). Heat-stable microbial metabolites, mainly enzymes and toxins, can also cause problems if migration takes place from a packaging material into a foodstuff. Hydrogen sulfide produced by sulfate-reducing bacteria and volatile metabolites (fatty acids) produced by many *Clostridium* spp. may cause organoleptic problems in the final products (Dyer, 1996; Harju-Jeanty and Vaatane, 1984; Robichaud, 1991).

The papermaker is constantly troubled with slimy or gelatinous accumulations that adhere to the inside of the pipe lines, chests, and screens and in particular on the exposed spiders of the cylinder machines and along the edge of the wire pit on Fourdrinier machines. A slippery feel on any of these surfaces is an indication of slime. Slime sticks firmly to the inside of the pipe lines, chests, and screens and also on the exposed spiders of the cylinder machines and along the edge of the wire pit on Fourdrinier machines. The deposit frequently grow to such size that they break loosely from their point of attachment and are carried along with the stock to the paper machine, where they cause clogging of the felts and wires and cause breaks on the paper machine. Mills try to keep slime under control by the use of toxicants and regular washing. Slime can build up in the paper mill system unnoticed; the first indication may be breaks on the machine and production of dirty paper. Careful examination of the machine and the use of slime measuring boards are the best indications of the slime buildup.

Slime deposits at critical points within the paper machine and cause serious operational problems (Sanborn, 1965; Blanco et al., 1996, 1997; Lindberg et al., 2001; Väisänen et al., 1989, 1994; Desjardins and Beaulieu, 2003; Rättö et al., 2005; Chaudhary et al., 1997). Slime buildup in paper-processing machines, caused by microbial biofilms, may cause significant economic losses, mainly from machinery-running problems in addition to quality problems in the end-product (Blanco et al., 1997) (Table 1.2). The true nature of slime and the causes of its formation are complex, and many factors interact to establish the necessary conditions for slime formation. As stated previously, paper mills, especially those employing increasingly closed processes and higher use of secondary fibers, have high nutrient levels as well as optimal temperature and pH ranges to support serious microbial proliferation. Many of these microorganisms develop slimy capsular materials around the cell. This capsular material enables the cells to attach to each other and to adhere to surfaces.

In general, slime contains consortia of species (Characklis and Cooksey, 1983; Geesey and Costerton, 1986; Geesey, 1982, 1994). Although the major microbial population in slime deposits is bacteria, fungi, yeast, and even protozoa, some authors suggest that the usual types of microorganisms that cause slime in industrial environments are the heavily encapsulated, fast-growing bacteria like *Pseudomonas*, *Aerobacter*, *Alcaligenes*, *Arthrobacter*, *Proteus*, *Bacillus*, and others (Lutey, 1972; Prendergast, 1948; Purkiss, 1970; Sanborn, 1965; Väisänen et al., 1994). Bacteria found most often in paper and board machine slime include species of *Sphaerotilus*, *Leptothrix*, *Flavobacterium*, *Clavibacter*, etc. (Purkiss, 1970). Fungi such as *Aspergillus*, *Cephalosporium*, and *Penicillium* are also found in slime (Geesey and Costerton, 1986; Hughes-van Kregten, 1988; Väisänen et al., 1994).

There are two groups of microbial population—primary slime former and secondary slime-former—in slime deposits (Safade, 1988). Primary slime formers cause the accumulation of slime by themselves. In this group, there are bacterial species (e.g., *Enterobacter agglomerans* or *Pseudomonas aeruginosa*) and yeasts (e.g., *Rhodotorula mucilaginosa*) (Blanco, 2003). These primary formers allow the growth of colonies of secondary microorganisms. Sulfate-reducing bacteria and *Penicillium* belong to this group (Blanco, 2003). Distinction between primary and secondary slime-formers is difficult because it is not easy to assess the importance of a single species in a slime-producing consortium. This is due to variations in

Table 1.2: Economical losses as a consequence of the microbial growth in the paper and board mills

Reduced production resulting from a larger number of web breaks, downtime for cleaning and maintenance of the machinery
Higher production cost due to a larger consumption of additives
Reduction of the equipment life caused by corrosion, scale, fouling, and plugging
Safety problems resulting from the presence of explosive and inflammable and lethal gases by inhalation
Drop in sales as a result of the loss of clients

Based on Blanco et al. (1997).

the concentrations of nutrients, physical conditions, presence of inhibitors and activators, seasonal changes, process cleanliness, and the nature of pulp stock (Blanco et al., 1996; Geesey, 1994; Johnsrud, 2000; Van Loosdrecht et al., 1995).

The slime may be homo- or heteropolysaccharides, but homopolysaccharide-based slimes are found to be more common. In many cases, the slime has been identified as levan, a polyfructan with beta (2,6) linkages with some branching through beta-2,1-linkage (Verhoef et al., 2002). This capsular material entraps debris such as fillers and fibers used in the mill to form the final deposits. If microbial growth remains unchecked for long, the built-up deposits may dislodge onto paper sheets, affecting quality and causing malfunctions in the machinery. In addition to good housekeeping practices and routine cleanups or boilouts, papermakers use biocides to control microbiological growth. But these efforts are often insufficient because the protective slime around microorganisms and throughout the deposit restricts the efficacy of biocide. Also, excessive biocides usage produces undesirable side effects such as odor, off-color development in the paper sheet, increased toxicity of mill effluent, and upsets in the waste treatment system.

With continuing pressures for improved production efficiency and sheet quality, the prevailing measures for the control of slime problem do not provide a satisfactory solution.

Standard biocides, because of their hazardous properties, are highly regulated substances worldwide. The regulatory considerations are having a profound effect for the pulp and paper industry, most notably in the United States, Canada, and Western Europe. The US Environmental Protection Agency's product registration process is costly and time-consuming. The Canadian process is comparable to that in the United States. The European Union's Biocidal Products Directive went into effect in 2000 and will eventually remove a large number of products from the market if they do not pass regulatory round-up or producers decide to not submit them for approval. In developing regions, regulatory climates vary widely. In several countries, regulations concerning biocide use are beginning to resemble those in the United States, Canada, Japan, and Western Europe. Alternative methods to conventional biocides are being investigated for slime control. Innovative ideas in enzyme production allow the commercial use of a green enzymatic biocide that replaces standard biocides in pulp and paper mills without negative effects on plant workers or the environment (Siika-aho et al., 2000; Torres et al., 2012; Bajpai, 2012). The use of the enzyme may completely eliminate slime from the system, or the enzyme may be employed in conjunction with biocides to lower the biocide dosages and indirectly reduce environmental problems.

The effects of slime deposits are generally referred to as biofouling. In principle, the processes underlying biofouling in a paper mill are the same as those typical of a wastewater treatment plant: the microorganisms form films and flocs, use organic carbon, and convert it into metabolic products and new biomass. In a wastewater treatment plant, this is a desired process that eliminates carbon from water while generating activated sludge; in a paper mill,

exactly the same process take place, but in this context, it constitutes biofouling (Flemming, 2002). According to Flemming et al. (2013), there is no “silver bullet” against biofouling in paper production. Effective countermeasures have to be based on holistic approaches.

Not much is known about the mechanisms that lead to biofilm formation in the wet-end part of the machine. The science of paper machine slime formation and control is only just beginning to be understood in detail. Corrosion caused by slime control chemicals is a very serious problem that has appeared in the past few years. Papermakers all over the world are very concerned about vapor phase corrosion on their paper machines and significant efforts are being made to solve this problem.

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Paper Machine Loops and Papermaking

The paper industry is moving toward more sustainable or environmentally friendly processes (Bajpai, 2010). As a result, most modern paper mills are operating a closed loop process water system that operates under neutral or alkaline conditions with an increased consumption of recycled fibers as raw material. Currently, more than 50% of the produced paper and board is derived from recycled fibers. Increased whitewater closure changes the environment and growth conditions for microorganisms in the papermaking process. These changes create conditions for microbial growth that result in deterioration of product quality. Microbiological growth can result in slime deposition, formation of volatile gases, or spoilage-producing acids that lead to material degradation. Quality of paper or paperboard is affected by sheet defects from microbiological deposition, odor complaints for paperboard from volatile fatty acid production, and reduction in sheet strength from fiber spoilage. Runnability of the papermaking process is affected by some of the following (Gudlauskis, 1996):

- Breaks from microbiological deposition
- Downtime to wash up or boilout deposition
- Deterioration of fiber or additives from degradation
- Reduced production capacity from screen plugging
- Downtime for repairs from deposit corrosion

Breaks and the corresponding downtime are the most common runnability problems. Safety becomes an issue when anaerobic bacteria produce toxic or explosive gases in stock or water chests. Slime deposition can make machine surfaces slippery, causing lost time accidents. Neighboring communities may complain of odor from volatile fatty acids or hydrogen sulfide being liberated from the sheet and/or process.

2.1 Papermaking

Figure 2.1 shows the general process of papermaking. After making the stock suspension of fibers and water, the slurry is pumped to the paper machine (Smook, 1992; Biermann, 1996). The slurry consists of approximately 99.5% water and approximately 0.5% pulp fiber. The exit point for the slurry is the “slice” or headbox opening. The fibrous mixture pours onto a traveling wire mesh in the Fourdrinier process, or onto a rotating cylinder in the cylinder machine (Biermann, 1996). The Fourdrinier machine is named after its French inventors, the Fourdrinier brothers, and is essentially a table over which the wire moves. Greater quantities of slurry released from the head box result in thicker paper. As the wire moves along the

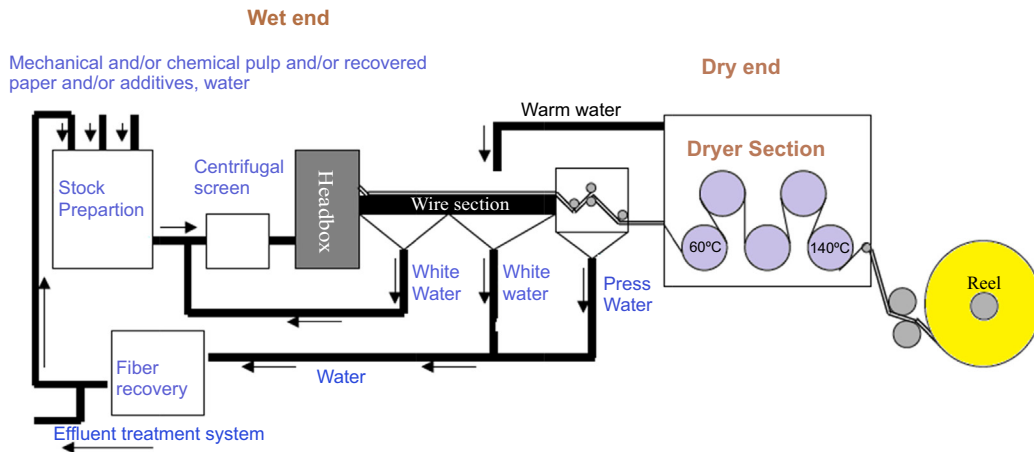


Figure 2.1

Process of papermaking. Based on *German Pulp and Paper Association (2005)*.

machine path, water drains through the mesh. Fibers align in the direction of the wire travel and interlace to improve the sheet formation. After the web forms on the wire, the task of the remaining portion of the paper machine is to remove additional water. Vacuum boxes located under the wire aid in this drainage.

One of the characteristics inherent in the performing of the sheet on a Fourdrinier paper machine is that all the water is removed through one side of the sheet. This can lead to differences in the sheet properties on one side as opposed to the other. This two-sided property increases as machine speed increases. In response to this, manufacturers developed twin wire and multiple Fourdrinier machines. Manufacturers of such equipment use different engineering designs that can be vertical or horizontal. After the paper web has completed its short forming distance, it continues along the second wire, losing water as it travels.

The next stop for the paper is the pressing and drying section where additional dewatering occurs (Smook, 1992; Biermann, 1996). The newly created web enters the press section and then the dryers. As the paper enters the press section, it undergoes compression between two rotating rolls to squeeze out more water. The extent of water removal from the forming and press sections depends greatly on the design of the machine and the running speed. When the paper leaves the press section, the sheet usually has about 65% moisture content. The paper web continues to thread its way through the steam-heated dryers, losing moisture each step of the way. The process evaporates many tons of water.

Paper will sometimes undergo a sizing or coating process. The web in these cases continues into a second drying operation before entering the calendaring stacks that are part of the finishing operation. Moisture content should be about 4–6% as predetermined by the mill.

If the paper is too dry, it may become too brittle. About 90% of the cost of removing water from the sheet occurs during the pressing and drying operations. Most of the cost is for the energy required for drying.

At the end of the paper machine, paper continues onto a reel for winding to the desired roll diameter. The machine tender cuts the paper at this diameter and immediately starts a new reel with the additional paper falling as an endless web.

For grades of paper used in the manufacture of corrugated paperboard, the process is now complete. For those papers used for other purposes, finishing and converting operations will now occur, typically off-line from the paper machine. These operations can include coating, calendaring, or super calendaring and winding.

The water that is disclosed from the wet paper sheet in the wire section is usually referred to as whitewater. This whitewater contains 3–18 mg/L of total nitrogen, 2–6 mg/L of total phosphorus, and approximately 500 mg/L dissolved organic carbon. The whitewater formed underneath the wire section is directed back to the stock preparation vessel via a closed loop system. [Figure 2.2](#) shows the circuits in the paper machine containing three main circulations for the water. The mills are decreasing fresh water consumption and are achieving partial or total water circuit closure. This results in reduction in losses of fines, fibers, additives, and enhanced dewatering from higher temperatures. Microbial life will be affected by the higher temperatures in the process water, by the increase in suspended solids, and dissolved and colloidal material ([Blanco, 2003](#)). If the mill only closes their circuits, the chemical oxygen demand values reach far above 10,000 mg/L, which affects the runnability and the quality of paper and board ([Geller, 1984](#)). There is significant differences in the concentration of volatile organics in a zero-discharge mill and an open paper mill ([Geller, 1984](#)). Several paper and board mills have solved this problem caused by the organic volatiles when closing the water circuits by installing a tertiary circuit for the water, where the water is taken back from the sewage plant after clarification and microbiological treatment ([Figure 2.2](#)). [Hamm and Schabel \(2007\)](#) did a chemical analysis of the whitewater before and after installation of a tertiary circuit with both anaerobic and aerobic treatment steps for circulation water in a board mill discharging 0 m³ water per ton board produced. After tertiary treatment, chemical oxygen demand, hardness, calcium, sulfate, chloride, volatile fatty acid, and conductivity reduced significantly. Most of the paper mills today are running at neutral or alkaline pH so that calcium carbonate as fillers in the paper/board can be used. It was previously recommended that paper machines should run at acidic pH to control microorganisms in the paper machine circuits ([Harju-Jeanty and Väättänen, 1984](#)).

It is hard to generalize when it comes to deposit formation from the different types of paper grades. Each type has its own raw material mix and the chemical composition. There are significant differences even within one variety of the graphical papers. The main difference between the paper grades is in the fiber material. Paper is produced from mechanical pulp, chemical pulp, and recycled fiber ([Smook, 1992; Biermann, 1996](#)). The additives and

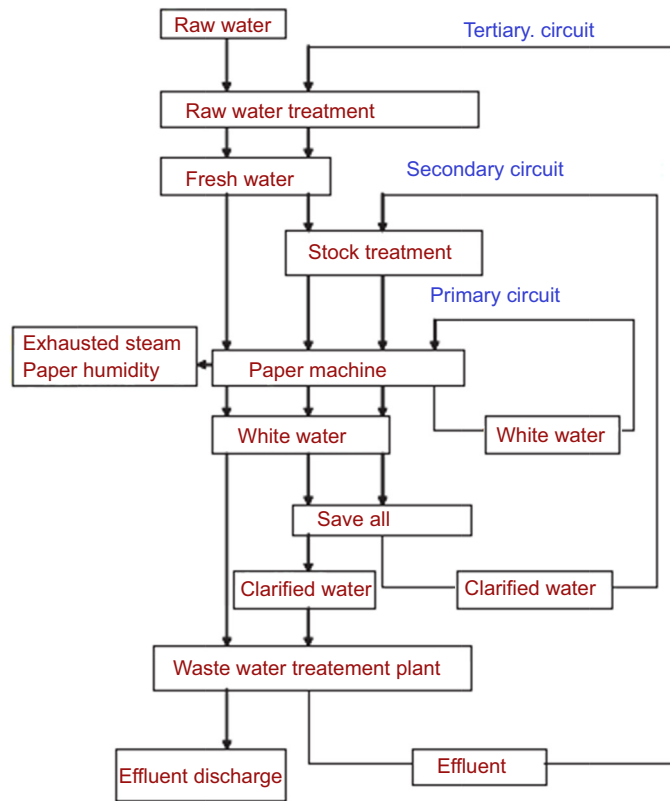


Figure 2.2

Papermaking production systems in three different main circulations. Based on [Blanco \(2003\)](#).

chemicals are added to achieve the desired paper properties. According to [Alen \(2007\)](#), the graphical paper grades account for about 30% of the world paper and board production. In some brands of paper, the sheet is mostly built up of several layers and is called board. Chipboard is the common name for board containing recycled fibers in one or several layers. Folding boxboard is the common name for a board used for the production of boxes. It is made up of multiple layers of chemical and mechanical pulp. This grade is made up of mechanical pulp between two layers of chemical pulp. The top layer is of bleached chemical pulp with an optional pigment coating. This is a low-density material with high stiffness and has a slightly yellow color, mainly on the inside. Linerboard made of virgin pulp is called *kraftliner*, whereas recycled linerboard is known as *testliner* ([Persson, 2004](#)). Corrugated board is made from semichemical pulp and also from recycled fibers. Corrugated board consists of one or more sheets of fluted paper adhered to one or more liner papers. The manufacturing process requires at least two layers of paper, very high humidity (steam), glue, and heating only, which is why corrugated is treated as an environmentally

friendly product. Several boards are made using variations of liner material, fluting medium, etc. (Persson, 2004; Alén, 2007; Biermann, 1996). Board is also produced in several bleached qualities. Tissue paper is made from bleached kraft, sulfite pulp, and also from recycled fibers. It is characterized by extreme lightness and transparency. Tissue paper is used to make napkins, bathroom tissue, paper towels, etc. (Biermann, 1996). Other types of specialty papers are glassine, greaseproof paper, bond paper, construction board, egg cartons, and other molded products. These papers contain their own special mixture of pulp and additives (Biermann, 1996).

As stated previously (see Chapter 1), recycled fiber is an important raw material for the paper industry. However, recycled paper fiber contains many items affecting deposit formation, thereby raising concerns about the hygienic quality of the final product (Gendron et al., 2012). Its use may also increase the rate of microbial growth because of increased nutrient concentrations in the water. Anaerobes also increase as the water system is closed. These issues are not just confined to systems using recycled paper, but experience shows that the overall organic burden increases more rapidly in such systems. It is assumed that the various steps in processing recycled paper such as grinding, bleaching, and deinking will inactivate most of the bacteria. Exposure to biocides and high temperatures during drying usually restricts the survival of spores. Increased deposit problems with recycled fiber is due to the reason that it consists of short fibers (fines) and is contaminated with deinking chemicals, glue, and other substances. These contaminants also lead to an increased consumption of chemical additives and require the addition of retention aids to maintain stable paper.

Paper recovery rates continue to increase year after year in most parts of the world (Bajpai, 2013). The recycling rate in Europe is currently 72.0%. Last year, it was 71.7%, according to a statement from the European Recovered Paper Council. In 2013, 13 European countries exceeded a 70% recycling rate; of the 11 European countries below 60%, eight significantly improved their performance compared with 2012 (<http://europe.paperrecyclingconference.com/Article>). Exports of recovered fiber from the United States to Asia have grown rapidly, representing a nearly three-fold increase since 2002. In many parts of the world, the collection of used paper is organized by independent companies, which sell it to paper mills. Recycled fiber can be used in many different paper grades, and the use is increasing every year. The quality demands of the raw material differ between the application fields and therefore the recovered paper must be sorted before processing. Textile fabric, pieces of wood, glass, plastic, and other foreign materials are mainly removed when sorting (Persson, 2004). Recovered paper can be divided into different quality grades. To facilitate handling and transport the paper is normally pressed into bales. The demands on reuse are getting greater in many countries. The disinclination for burning waste products has increased, making it difficult to find suitable dumping grounds and it is considered to be a good material to recycle. The greatest admixture is made in tissue paper, but the percentage in other grades

Table 2.1: Functional additives used in papermaking

Internal size
Resistance to water penetration
rosin sizing with alum, alkylketone dimer (AKD), alkyl succinic anhydride (ASA)
Fillers
Increase opacity, brightness and basis weight
clay, calcium carbonate, talc, titanium dioxide
Dry strength additives
Increase hydrogen bonding
polyacrylamides, starch, guar gum, (carboxy)methyl cellulose
Wet strength resins
Promote covalent fibre–fibre bonding
urea formaldehyde (UF), melamine-formaldehyde (MF), glyoxal-polyacrylamide copolymers, epoxidized polyamine-polyamide
Dyes and brighteners
Impart color/brightness
basic/acid dyes, direct dyes, fluorescent brightening agents
Specialty chemicals
Flame retardants, anti-tarnish chemicals etc.

Based on [Rice \(2001\)](#), [Hipolit \(1992\)](#), [Biermann \(1996\)](#).

increases constantly. Kitchen rolls and toilet paper are examples of tissue products that can be produced totally from recycled fiber.

Paper is made primarily from cellulose fibers, but it also contains various amounts of a variety of additives. Without the use of additives, it is not possible to achieve the optimum properties required for the different paper qualities ([Alén, 2007](#); [Persson, 2004](#); [Biermann, 1996](#)). The additives can be divided into two groups ([Tables 2.1 and 2.2](#)) ([Rice, 2001](#); [Hipolit, 1992](#); [Biermann, 1996](#)).

Functional additives: Added to give the paper new properties.

Processing aids: Make the functional additives attach to the fibers or affect the runnability of the machine.

Many of the papermaking additives contribute to deposit formation. According to [Kanto Öqvist \(2008\)](#), this must be considered when analysis and treatments are done to improve paper quality and runnability of the paper/board and paper. In most cases, it would seem difficult to achieve the desired paper without chemical additives. Fillers improve the paper properties and are less expensive than the fiber. Additives prevent the paper from absorbing water (size, hydrophobation agent), give strong fiber bonds (dry strength agent) and water proof bonds (wet strength agent), bind fine material, and increase production (retention and drainage agent) and opacity and brightness.

Table 2.2: Process chemicals or aids for papermaking

Formation aids

Promotes dispersion of fibers

- water-soluble linear polyelectrolytes of ultra high molecular weight (anionic polyacrylamides), guar gum

Retention and drainage aids

Keeps material on the sheet and increases water removal on the wire

- high charge density polyelectrolytes (polyethylenimine, poly(diallyldimethyl ammonium chloride)), high m.w. polyacrylamides, polyethyleneoxide, starches, gums, alum, aluminum polymers

Deposit control agents

Used to control inorganic or organic deposits

- chelants (EDTA, NTA, DTPA), polyphosphates, polyacrylates, phosphonates, non-ionic surfactants, polypropylene

Defoamers

Used to control entrapped air

- alkylpolyesters, polydimethylsiloxanes, oligomers of ethylene oxide (EO) or polypropylene oxide (PO), hydrocarbon or polyethylene waxes, fatty alcohols, fatty acids, fatty esters, ethylenebisstearamide (EBS)

Biocides

Reduces slime from microorganisms

- quaternary ammonium salts, methylene bis-thiocyanate, dibromonitrilepropionamide, glutaraldehyde, isothiazolin

Other additives

pH control agents, corrosion inhibitors

Based on *Rice (2001)*, *Hipolit (1992)*, *Biermann (1996)*.

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Microbial Problems in Papermaking and Consequences

Paper mills are open systems that provide an ideal environment for microbes to grow and reproduce (Kolari et al., 2003). Microbes are present in every paper machine, and their presence in the process is unavoidable. The reduction of fresh water consumption and closing up of water cycles causes the buildup of dissolved organic material used by microbes as nutrition. This, together with increased use of recycled fibers and the move from acidic to neutral or alkaline paper manufacturing processes, are factors that have increased the amounts of microbes in paper machine systems, and also the extent of problems related to these microbes (Blanco et al., 1997). The increase in pH has been correlated with increased slime formation, higher numbers of anaerobes, formation of malodors, graying of thick stock, flotation in sedimentation tanks, and elevated numbers of holes and disruptions to paper quality (Robertson, 2009; Alén, 2007; Blanco et al., 1996). Slade et al. (2004) has reported that paper machine waters and wastewaters contain insufficient nitrogen and phosphorus to satisfy bacterial growth requirement in comparison to many other industries.

There are several types of microbes present in paper machine, including spore-forming aerobic bacteria, nonsporulating aerobic bacteria, and anaerobic bacteria. Molds, yeasts, and algae may also occasionally be present (Väisänen et al., 1998). The most common types of microbes found in the paper machine wet end are aerobic bacteria belonging to the genera *Bacillus*, *Burkholderia*, *Pantoea*, *Ralstonia*, and *Thermomonas* (Kolari, 2003). The factors affecting the growth of microbes are listed in Table 3.1. If the growth of microbes is not controlled, problems can occur in the papermaking process. These include runnability problems, poor end-product quality, and deterioration of the raw material (Edwards, 1996). Bad odor and premature wearing of machine parts such as felts are also common problems caused by excessive microbial growth (Ludensky, 2003). Table 3.2 presents a list of microbial problems encountered in papermaking. Slime deposits can cause plugging and fouling of felts, showers, and pipes, resulting in paper machine runnability problems. It has been estimated that 10–20% of paper machine downtime is caused by slime problems (Blanco et al., 1996). Flemming et al. (2013) have reported that the major cost factors associated with slime formation are downtime and cleaning costs. Much of the cost is the result of breakdowns caused by lumps of slime dropping onto the moving screen, on which the water is separated from the pulp, and causing holes. It has been estimated that depending on the size of the plant, the process and the quality of the paper, a breakdown can cost between US \$2000 and \$10,000. If the breakdown happens twice daily, the efficiency of the

Table 3.1: Factors affecting the growth of micro-organisms

Microorganisms	Optimal temperature	Optimal pH	Oxygen	Light	Water
Algae	<35 °C for green algae	4.5–9.0	O ₂ needed to start photosynthesis	Essential	Essential
Fungi	22–30 °C Molds T _{max} = 65 °C Yeast T _{max} = 45 °C	5.0–6.0 4.0–5.0	Molds >4 ppm Yeast no effect	Could affect spore germination	Nonessential
Bacteria	Psychrophiles 15 °C Mesophiles 35 °C Thermophiles 55 °C	6.5–7.5	Aerobic >4 ppm Anaerobic <4 ppm Facultative: no effect	No effect	Essential
Protozoa	20 °C	6.5–7.5	Aerobic >2 ppm Anaerobic <2 ppm	No effect	Nonessential

Based on Blanco et al. (1997).

Table 3.2: Microbial problems in a paper mill

Production
Slime deposits Breaks Corrosion Felt plugging Odors Reduced flows
Quality
Holes and spots Malodor of paper Discoloration Brightness losses Increased dirt count Microbial contamination (hygienic problems)
Raw Materials
Fiber degradation Additive contamination Malodor formation Reduction in strength properties Changes of viscosity of pigment slurries and starch solution Fouling of probes Impairment of functionality of flocculants
Waste Water
Bulking sludge Floating sludge, scum Odor problem

Based on Kiuru (2011) and Flemming et al. (2013).

plant is significantly reduced. Not all breaks are due to biofouling, but one to two of five are probably related to microbial biomass. In addition, the costs for downtime during cleaning and those associated with labor and chemicals have to be considered. The possibility of damaged product and high maintenance costs should also be taken in to consideration (Flemming et al., 2013). Haapala et al. (2010) noted that more than 60% of the web breaks were caused by holes or deposits on paper web. Most of the deposits and edges of the holes contained bacterial DNA, indicating microbial involvement in the formation of the deposit (Haapala et al., 2010).

Several microbial problems have been encountered in papermaking (Blanco, 2003; Kolari, 2007; Blanco et al., 2004; Kanto Öqvist, 2008; Väisänen et al., 1989, 1991, 1998; Suihko and Stackebrandt, 2003; Priha et al., 2004; Johansson et al., 2001; Suominen et al., 1997; Stenfors Arnesen et al., 2008; Hansen and Hendriksen, 2001; Hansen et al., 2001; Ullah, 2011; Johnsrud, 2000; Jokinen, 1999; Keedy, 1988; Schenker and Gould, 1998; Schenker et al., 1998; Von Holy, 1985; Videla, and Characklis, 1992; Mattila et al., 2002; Harju-Jeanty and Vääänen, 1984; Vääänen and Niemelä, 1983). These are:

- Biofilm and stains/spots or holes in paper product
- Growth of micro-organisms on additives
- Malodor formation
- Quality of the paper products
- Microbially influenced corrosion

3.1 Biofilm and Stains/Spots or Holes in Paper Products

“Biofilm” is an expression for a variety of manifestations of microbial aggregates. Biofilms are the oldest and most successful form of life on Earth, with fossils dating back 3.5 billion years, and representing the first signs of life on Earth (Schopf et al., 1983). Aggregation and the association to surfaces offer considerable ecological advantages for micro-organisms (Characklis, 1973, 1981; Characklis and Cooksey, 1983; Characklis et al., 1990a,b; Costerton et al., 1978, 1981, 1987, 1992, 1994, 1995; Costerton and Lappin-Scott, 1989; Costerton and Lashen, 1984; Flemming, 2008). Almost all surfaces in nonsterile environments that offer sufficient amounts of water are colonized by biofilms. This happens even at very high pH values, high temperatures, pressure, high salt concentrations, and radiation intensities (O’Toole et al., 2000; Flemming, 2008). Biofilms are involved in the biogeochemical cycles of almost all elements and are the carriers of the environmental “self-purification” processes. The microbes on the surfaces convert particulate or dissolved nutrients from the water phase and/or from their support into metabolites and new biomass (Satpathy, 1999; Schopf et al., 1983; Wimpenny, 2000; Decho, 1990, 2000). This is the principle of biofiltration systems used in wastewater purification and many other biotechnological applications (Flemming and Wingender, 2003). Of all forms of life, microbial biofilms are present everywhere and have the highest survival potential. Biofilms, however, can occur in the wrong place and at the

wrong time. In that case, they are addressed as biofouling. It is observed in many different fields ([Flemming, 2002](#); [Henderson, 2010](#)):

- Ship hulls
- Oil
- Automobile
- Steel
- Paper production
- Food
- Beverage industries
- Water desalination
- Drinking water treatment, storage, and distribution

Microbes can also cause several problems in cooling towers ([Kiuru, 2011](#)). The biofilms that are formed in cooling towers lead to an increase in frictional fluid resistance and heat-transfer resistance in power plant condensers and process heat exchangers. Biofilms also produce premature wearing out of process equipment and increase the need for replacement ([Ludensky, 2003](#)). If care is not taken over mixing and the correct ventilation of chests, extensive growth of anaerobic bacteria may cause production of volatile fatty acids, which cause bad odors. sulfate-reducing bacteria use volatile fatty acids as nutrients and produce hydrogen sulfide. Sulfuric acid is also formed, which causes corrosion of the surfaces of machine parts ([Blanco et al., 1996](#)). Microbes use various additives—starch, retention, and sizing agents—as nutrients, which results in total or partial loss of their activity. Slurries containing starch provide very good growth medium for different micro-organisms and the biocidal treatments of slurries, especially mineral ones, when inaccurate, may lead to the total spoilage of them. Long storage of pulp without proper microorganism control can also lead to the decay of fiber material. Microbial spoilage of raw material and additives can cause difficulties in web formation and negatively affect the strength properties of the final product. Detached parts of slime deposits can find their way into the final product, where they can result in spots and holes ([Blanco et al., 1996](#)). Unchecked microbial growth results in a variety of problems in coating and raw materials related to coating as well. The problems are found to be similar to wet-end: viscosity increase in pigment slurries as the dispersant is degraded, pH drop, loss in brightness and odor problems from anaerobic growth, and starches and protein lose viscosity as they are degraded ([Woodward, 2009](#)). Free-swimming bacteria, even when present in process water in large numbers, do not always affect machine operation or end-product quality. Problems frequently arise when bacterial cells become attached to the surfaces of tank walls, pipes, or other paper machine parts and form colonizations ([Väisänen et al., 1998](#)). These colonizations are called biofilms (scientific) or slimes (in practice). [Bunnage et al. \(2000\)](#) have described the primary characteristics of biofilm and general paper machine deposits ([Tables 3.3 and 3.4](#)).

The buildup of a biofilm is often shown using a five-stage model ([Table 3.5](#); [Figure 3.1](#)) ([Breyers and Ratner, 2004](#); [Chmielewski and Frank, 2003](#); [Donlan and Costerton, 2002](#); [Hall-Stoodley](#)

Table 3.3: Characteristics of biofilms

Characteristic	Range
Water content	High (70–95%)
Organic content	High (70–95% dry weight)
Bacterial content	High (10^6 cells/g wet weight)
Biological activity	High
Inorganic content	Low

Based on *Bunnage et al. (2000)*.

Table 3.4: Characteristics of paper machine deposits

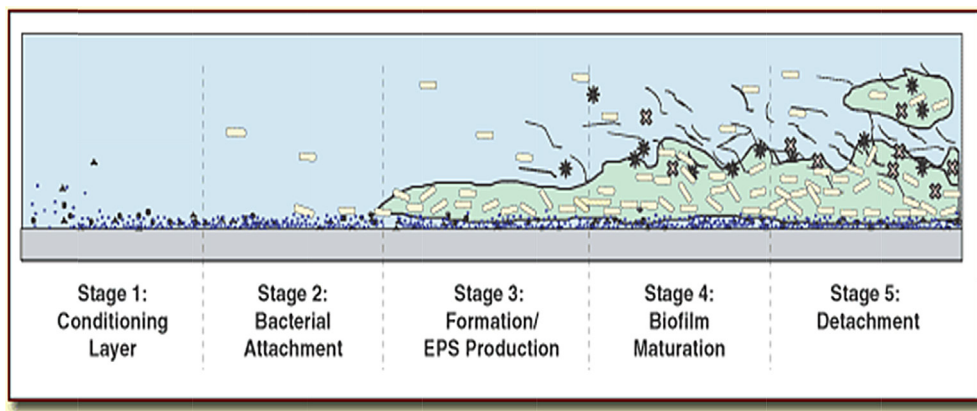
Characteristic	Range
Microbiological	Filamentous bacteria, unicellular bacteria, fungi
Particle materials (organic and inorganic)	Calcium carbonate, clay fines, fibers, pitch, chemical deposition
Dissolved substances	Minerals, sodium, chlorides

Based on *Bunnage et al. (2000)*.

Table 3.5: Model of biofilm formation on paper machine surfaces

Stage 1: Conditioning layer
Stage 2: Bacterial attachment
Stage 3: Biofilm formation/exopolysaccharides synthesis
Stage 4: Biofilm maturation
Stage 5: Dislodgement/detachment

Based on *Schenker et al. (1998)*.

**Figure 3.1**

Biofilm formation in paper machine systems. *Reproduced with permission Davis and Casini (2003)*.

and Stoodley, 2002; O'Toole et al., 2000; Verstraeten et al., 2008; Davis and Casni, 2003). Breyers and Ratner (2004) have reported processes which govern the formation of biofilms:

1. Preconditioning of the adhesion surface either by macromolecules present in the bulk liquid or intentionally coated on the surface
2. Transport of planktonic cells from the bulk liquid to the surface
3. Adsorption of cells on the surface
4. Desorption of reversibly adsorbed cells
5. Irreversible adsorption of bacterial cells on the surface
6. Production of cell–cell signalling molecules
7. Transport of substrates to and within the biofilm
8. Substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm. These processes are accompanied by cell growth, replication, and production of extracellular polymeric substances (EPS)
9. Removal of biofilm by detachment or sloughing

Growth of biofilm is regulated by several physical, chemical, and biological processes. Attachment of a cell to a substrate is termed adhesion; cell-to-cell attachment is termed cohesion. The mechanisms behind these forms of attachment finally determine the adhesive and cohesive properties a biofilm will show (Garrett et al., 2008).

The attachment of micro-organisms to surfaces and the subsequent biofilm development are very complex processes. These are affected by several parameters (Table 3.6). Normally, attachment occurs most readily on surfaces that are rougher, more hydrophobic, and coated by surface conditioning films (Chae et al., 2006; Donlan, 2002; Millsap et al., 1997; Oulahal et al., 2008; Patel et al., 2007; Simoes et al., 2008; Watnick and Kolter, 1999).

Fletcher (1980) described the microbial adhesion to surfaces as a process of three stages:

- (1) Adsorption of microorganism on a collector surface, i.e., substrate (deposition)
- (2) Attachment between microorganism and a collector; this often involves the formation of polymer bridges between the microorganism and collector
- (3) Colonization and division of micro-organisms on the collector's surface

Table 3.6: Factors important in cell attachment, biofilm formation, and development

Adhesion surface	Bulk	Fluid cell
Texture or roughness	Flow velocity pH, temperature	Cell surface hydrophobicity
Hydrophobicity		Extracellular appendages
Surface chemistry		Extracellular polymeric substances
Charge	Cations	Signaling molecules
Conditioning film	Presence of antimicrobial products	
	Nutrient availability	

Based on Donlan (2002).

This type of profile is limited when considering the intimate processes of cell–substrate/ cell–cell interaction.

Characklis and Marshal (1990) later described a process that involved the formation of an initial conditioning layer, reversible and irreversible adhesion of bacteria, and ultimately detachment of bacterial cells from a mature biofilm for subsequent colonization. The conditioning layer can be composed of organic or inorganic particles. It is the foundation on which a biofilm grows. Anything that may be present within the bulk fluid can settle onto a substrate through gravitational force or movement of flow and become part of a conditioning layer. This layer modifies the substrate and facilitates accessibility to bacteria. The substrate provides anchorage and nutrients and enhances growth of the bacterial community. By the interactions between the conditioning layer and the substrate, surface charge, potential, and tensions can be changed. At first, planktonic microbial cells are transported from bulk liquid by physical forces or by bacterial appendages (flagella) to the conditioned surface. A fraction of the cells reaching the surface gets reversibly adsorbed. Environmental variables such as surface functionality, bacterial orientation, available energy, temperature, and pressure conditions contribute to bacterial adhesion. The bacteria detach from the surface if repulsive forces are larger than the attractive forces. In all probability, this occurs before conditioning of a substrate. The activation energy for desorption of bacteria is low and therefore it is likely to occur. This shows the weakness of the bonds. Physical forces that are associated to bacterial adhesion include the van der Waals forces, steric interactions, and electrostatic (double layer) interaction. These are collectively known as the Derjaguin, Verwey, Landau, and Overbeek, or DVLO, forces (Rutter and Vincent, 1980). This theory has been used to describe the net interaction between a cell and a flat surface as a balance between attractive (van der Waals interactions) and repulsion interactions from the overlap between the electrical double layer of the cell and the substrate (repulsive because of negative charges of the cells) (Hermansson, 1999). These are long-range forces known as physical interactions. An extended Derjaguin, Verwey, Landau, and Overbeek theory takes into consideration hydrophobic/ hydrophilic and osmotic interactions and has also been described in terms of thermodynamic interaction (Chang and Chang, 1992; Gallardo-Moreno et al., 2002). Several reversibly adsorbed cells remain immobilized in real time and get irreversibly adsorbed. The physical appendages of bacteria—flagella, fimbriae, and pili—overcome the physical repulsive forces of the electrical double layer (De Weger et al., 1987). Afterwards, the appendages make contact with the bulk lattice of the conditioning layer and stimulate chemical reactions such as oxidation and hydration and consolidate the bacteria–surface bond (Ganesh and Anand, 1998).

Liu et al. (2004) have reported that microbial adhesion strongly depends on the hydrophobic–hydrophilic properties of interacting surfaces. The stationary cells divide and the daughter cells spread outward and upward from the attachment point to form clusters (Hall-Stoodley and Stoodley, 2002). These interactions and growth within the developing biofilm form into a

mushroom-like structure. This structure allows the passage of nutrients to bacteria deep within a biofilm. After an initial lag phase, a rapid increase in microbial population is observed. The rapid growth occurs at the expense of the surrounding nutrients from the bulk fluid and the substrate. At this stage, the physical and chemical contribution to the initial attachment ends and the biological processes begin to take over. Excretion of polysaccharide intercellular adhesion polymers and the presence of divalent cations interact to form stronger bonds between cells (Dunne, 2002). Differential gene expression between the planktonic and sessile bacterial states is in part associated with the adhesive requirements of the microbial population. The production of surface appendages is inhibited in sessile species because motility is restricted. Simultaneously, expression of a number of genes for the production of cell surface proteins and excretion products increases. Surface proteins allow the transport of extracellular products into the cell and excretion materials out of the cell (Hancock et al., 1990). The structure of many gram-negative bacterial polysaccharides is relatively simple. These polysaccharides contain either homopolysaccharides or heteropolysaccharides and impart mechanical stability and are crucial for biofilm adhesion and cohesion and also avoidance from severe dynamic environmental conditions (Sutherland, 2001a, b).

Hall-Stoodley and Stoodley (2002) identified the differences in gene expression of planktonic and sessile cells; as many as 57 biofilm-associated proteins were not detected in the planktonic profile. The stationary growth phase describes a phase in which the rate of cell division equals the rate of cell death. At high cell concentrations, a series of cell-signaling mechanisms are employed by the biofilm; this is collectively termed quorum sensing (Bassler, 1999), which describes a process in which a number of auto-inducers are used to stimulate genetic expression of both mechanical and enzymatic processors of alginates, which form a fundamental part of the extracellular matrix. The death phase sees the breakdown of the biofilm. Enzymes are produced by the community itself, which works on the polysaccharides holding the biofilm together, actively releasing surface bacteria for colonization of fresh substrates. Alginate lyase produced by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, N-acetyl-heparosan lyase by *Escherichia coli*, and hyaluronidase by *Streptococcus equi* are examples of the enzymes used for the breakdown of the biofilm matrix (Sutherland, 1999a,b,c). Concurrently, the operons coding for flagella proteins are up regulated so that the micro-organisms have the apparatus for motility, and the genes coding for a number of porins are down-regulated, thus completing a genetic cycle for biofilm adhesion and cohesion.

3.2 Extracellular Polymeric Substances

A feature all biofilms have in common is that the organisms are embedded in a matrix of microbial origin, consisting of EPS (Table 3.7). Microbial EPS stands for extracellular polymeric substances, extracellular polysaccharides, exopolysaccharides, or exopolymers. EPS is either associated with the microbial cell wall in the form of capsules or completely dissociated from the microbial cell. EPS has been characterized extensively over the past 2 decades (Sutherland,

Table 3.7: Extracellular polymeric substances (EPS)

EPS as a structural element of biofilms	Development of microconsortia
	Concentration gradients
	Retention of extracellular enzymes
	Polysaccharide–exoenzyme interactions
	Prevention of loss of lysed cell components
	Convective mass transport through channels
	Pool of genes, easy horizontal gene transfer
	Matrix for exchange of signaling molecules
	Light transmission into biofilm depth

Based on Flemming (2002).

1990, 1994, 1998; Neu and Lawrence, 1999; Wingender et al., 1999; Costerton et al., 1978, 1981, 1992; Nielsen et al., 1997; Oliveira et al., 1994; Villain-Simonnet et al., 2000). Polysaccharides are found to be the major component of EPS (Vu et al., 2009) and they generally make up >50% of the total EPS (Frølund et al., 1996; Wingender et al., 1999a,b; Wingender et al., 2001), but in some cases, proteins dominate the EPS composition (Flemming and Wingender, 2010). If only one type of sugar is present, EPS is defined as homopolysaccharides. If two or more different sugars are present, then EPS is defined as heteropolysaccharides. EPS is built of linear or branched repeating units of two–seven different or same sugars. Most microbial polysaccharides are assumed to be heteropolysaccharides. In EPS, proteins, nucleic acids, and amphiphilic compounds including phospholipids are also present. Basically, EPS is composed of carbohydrates, but they can be substituted with both organic and inorganic substituents in addition to the various sugars. Several reports have shown wide range of sugar components in the polysaccharide moiety of EPS. D-glucose, D-galactose, D-mannose, L-fucose, and L-rhamnose are often found within EPS. These sugars are present in the pyranose form. EPS can also be characterized by their polyanionic nature because of the presence of uronic acids (Väisänen et al., 1994; Rättö et al., 2001). D-glucuronic acid is found to be the major sugar. Galacturonic and mannuronic acid are not found to be much. Noncarbohydrate ester–linked organic and inorganic substituents are also found to be present in EPS besides various carbohydrates. Pyruvate ketals are often observed as an organic substituent apart from ester-linked substituents. Hernandez-Mena and Friend (1993) found that the exopolysaccharides of paper machine biofilms were dominated by heteropolysaccharides that incorporated sugars such as glucose, mannose, galactose, rhamnose, fucose, and guluronic acid. But they did not report absolute amounts. Verhoef et al. (2005) analyzed the EPS of microbial isolates from paper mill slime deposits and observed four groups of EPS polysaccharide by principal component analysis:

- A group of *Enterobacter* with colanic acid (also found by Väisänen et al. (1994) and Rättö et al. (2006))
- An EPS from *Methylobacterium* sp. with high galactose and pyruvate levels
- Two groups of EPS associated with *Bacillus* sp. and *Klebsiella* sp. that were rich in mannose, glucose, and galactose

Different varieties of EPS structures are found because of the presence of different types of sugars and substituents in different forms and configuration linked to each other with different types of glycosidic linkages in different sequences (Sutherland, 2001a,b, 2007; Sand and Gehrke, 2006; Frølund et al., 1996; Whitchurch et al., 2002; Böckelmann et al., 2006).

The lack of accurate data with respect to the type of uronic acid present and the massive variation in sequence, linkage type and α or β -anomeric configuration make it impossible to directly translate these sugar compositions in chemical fine structures of EPS. Furthermore, important substituents like pyruvate ketals, succinyl half esters, and O-acetyl groups are often not taken into account.

Uronic acids and nonsugar substituents also contribute to the physical properties of the EPS. Pyruvate ketals, succinyl half esters, and uronic acids contribute to the polyanionic nature of most EPS and in turn have a large impact on the EPS's physical properties by making interaction possible via divalent cation bridges. O-acetylation can result in localized hydrophobic regions that are very important for the physical behavior of the EPS (Sutherland, 1994, 1999a,b,c; Wingender et al., 1999a,b).

The EPS also consists of extracellular DNA. In *Myxococcus xanthus*, DNA was found to strengthen the mechanical stability of the matrix (Wingender et al., 1999a,b; Hu et al., 2012). The exact composition of EPS is highly variable. Table 3.8 shows the general composition of bacterial EPS.

Nielsen et al. (1997) studied the EPS composition for biofilms from different engineered systems and found protein to be the largest fraction. Dignac et al. (1998) also observed that in

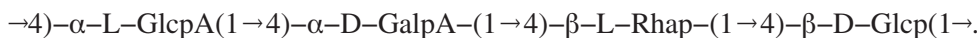
Table 3.8: General composition of bacterial EPS

Polysaccharides
Components: Monosaccharides, uronic acids, amino sugars Linkage: Glycosidic bonds Structure: Linear, branched Substituents: O-acetyl, N-acetyl, succinyl, pyruvoyl, sulfate, phosphate
Proteins
Components: Amino acids Linkage: Peptide bonds Structure: Linear Substituents: Oligosaccharides (glycoproteins), lipids (lipoproteins)
Nucleic acids
Components: Nucleotides, fatty acids, glycerol, phosphate, ethanolamine, serine, choline, sugars Linkage: Phosphodiester bonds, ester bonds Structure: Linear side chains

Based on Wingender et al. (1999).

EPS, protein predominated. Because protein has a high content of negatively charged amino acids, protein is more involved than sugars in electrostatic bonds with multivalent cations, which are a key factor in stabilizing aggregate structure. EPS production by the microbial population is influenced by the composition and availability of nutrients. Bura et al. (1998) reported that in sequencing batch reactors containing sludge growing on synthetic wastewater, the ratio of carbon:nitrogen:phosphorous was affected by hydrophobicity, surface charge, and the EPS composition of microbial flocs. Under phosphorous-depleted conditions, there was an increase in uronic acids and DNA in the EPS, but a decrease in the surface charge of the microbial flocs. The accumulation of heavy metals in the EPS matrix has been attributed to uronic acids (Väisänen et al., 1994). Polymer production was increased under low-phosphate and low-nitrogen conditions in batch cultures of a methanogenic bacterium and it was proposed that carbon utilization shifted toward EPS production when the carbon:nitrogen and/or carbon:phosphorous ratio was increased (Veiga et al., 2012). Other factors—such as shear forces and the quantity and composition of nutrients—also affect EPS composition and cohesion (Flemming et al., 2013).

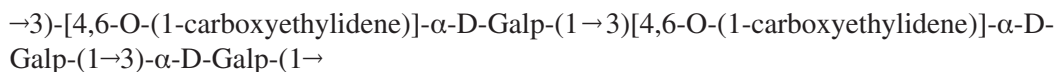
The percentage of various sugars in EPS in different paper grades furnish has been reported (Grant, 1998). Verhoef et al. (2002) have purified EPS from bacteria *Brevundimonas vesicularis*, which was isolated from a paper mill. Chemical, mass spectrometry, and nuclear magnetic resonance experiments showed that *B. vesicularis* sp. produces a linear exopolysaccharide without nonsugar substituents containing a tetrasaccharide-repeating unit with the following structure:



The novel EPS consists of only one distinct homolog population with a molecular weight distributed around 2000–4000 kDa and an intrinsic viscosity of around 0.5 dL/g. The novel EPS contains a linear backbone consisting of four sugar residues. In the terms of weight and volume, EPS represents the major structural component of biofilms, being responsible for the interaction of microbes with each other as well as with interfaces (Flemming, 2002; Neu et al., 2001; Hoyle et al., 1990; Corpe, 1980; Costerton et al., 1978; Dudman, 1977; Srinivasan et al., 1995). Slime binds in soft and viscous masses, which hook onto the sections of the paper machine where the amount of flow is not sufficiently powerful to dislodge them. These masses increase in volume until they fall off under their own weight and contaminate the pulp.

Bacteria belonging to the *Methylobacterium* sp. are known generally to be pink-pigmented bacteria that produce so-called pink slime. This slime-forming bacterium was isolated from a Finnish paper machine and its exopolysaccharide (EPS) was produced on laboratory scale by Verhoef et al. (2003). Sugar compositional analysis revealed a 100% galactan (EPS). However, Fourier transform infrared spectroscopy showed a very strong peak at 1611 cm⁻¹, revealing the presence of pyruvate. Analysis of the pyruvate content revealed that, based on the sugar composition, the EPS consists of a trisaccharide repeating unit consisting of

D-galactopyranose and [4,6-O-(1-carboxyethylidene)]-D-galactopyranose with a molar ratio of 1:2, respectively. Both linkage analysis and 2D homo- and heteronuclear ^1H and ^{13}C nuclear magnetic resonance spectroscopy revealed the following repeating unit:



By enrichment cultures from various ground and compost heap samples, a polysaccharide-degrading culture was obtained that produced an endo acting enzyme able to degrade the EPS described. The enzyme hydrolyzed the EPS to a large extent, releasing oligomers that mainly consisted out of two repeating units.

Most research has been conducted to identify the species present in EPS. Many different genera and species have been found, the most important of which are: *Flavobacterium*, *Enterobacter*, *Pseudomonas*, *Bacillus*, *Klebsiella*, *Sphaerotilus*, *Citrobacter*, and *Burkholderia cepacia* (Väisänen et al., 1994, 1998; Blanco et al., 1997; Safade, 1988; Klahre et al., 1997; Rättö et al., 1998; Oppong et al., 2000, 2003; Mattila-Sandholm and Wirtanen, 1992; Johnsrud, 1997; Chaudhary, 1992; Chaudhary et al., 1997; Pellegrin et al., 1999). Only sugar composition data are available; there is no clear information available about the chemical fine structure of EPS produced by these bacteria. Väisänen et al. (1994) analyzed different mixed EPS samples isolated from board machine slimes. They found different types of sugars, other than glucose derived from cellulosic fibers and starch used as raw material. Rhamnose, galactose, and mannose were the major sugars found within these samples. These researchers also determined the neutral sugars composition of EPS produced by several bacterial isolates from the same board machines grown on defined media. These bacterial isolates included *Klebsiella pneumonia*, *Enterobacter agglomerans*, *Pseudomonas* sp., *Flavobacterium* sp., *Clavibacter michiganese*, and *Bacillus licheniformis*. *K. pneumonia* produced an EPS consisting of rhamnose, galactose, and mannose; *E. agglomerans* produced an EPS that mainly consisted of fucose and galactose; *Pseudomonas* sp. EPS showed rhamnose, galactose, and mannose as the main sugar moieties of *Flavobacterium* sp.; *C. michiganese* and *B. licheniformis* EPS was composed of mainly mannose and galactose. All of the slime samples and EPS showed the presence of uronic acid; however, no distinction between galacturonic, glucuronic, and mannuronic acid was made.

Rättö et al. (1998) conducted similar studies on slime samples collected from paper machines. In these studies, rhamnose, galactose, and mannose were also found to be the main sugar moieties of the different EPS present. Both *Pseudomonas* sp. and *Citrobacter* sp. were isolated from the slime samples. The EPS produced by the *Pseudomonas* sp. mainly consisted of glucose, whereas the EPS produced by *Citrobacter* sp. mainly consisted of galactose, glucose, and mannose. In another study, Rättö et al. (2005) isolated polysaccharide-producing bacteria from slimes collected from two Finnish and one Spanish paper mill and characterized the exopolysaccharides produced by 18 isolates. Most of the isolates, selected on the bases of

slimy colony morphology, were members of the family Enterobacteriaceae, most frequently belonging to the genera *Enterobacter* and *Klebsiella* including *Raoultella*. All of the exopolysaccharides analyzed showed the presence of charged groups in the form of uronic acid or pyruvate revealing the polyanionic nature of these polysaccharides. Further results of the carbohydrate analysis showed that the EPS produced by nine of the enterobacteria was colanic acid.

Lindberg et al. (2001) grew the biofilms of different *Burkholderia cepacia* strains in paper mill whitewater-simulating conditions on glass slides or stainless steel coupons. The sugar content and composition of the biofilms were analyzed and compared with the sugar composition of paper mill slimes. Acid methanolysis followed by gas chromatography showed that *Burkholderia* was the major biofilm producer in pure culture, producing up to 50 μg of biofilm sugar cm^{-2} in 5 days in rich medium and 10 μg in paper mill simulating medium. A mixture of simulated paper mill water with a culture medium yielded more biofilm (100 $\mu\text{g cm}^{-2}$) than either of the media alone, so the biofilm accumulation was not proportional to the available substrate. More biofilm accumulated on stainless steel coupons than on glass slides, and the steel-coupon biofilms contained slightly more uronic acids. The biofilm sugars contained mainly galactose, glucose, mannose, and rhamnose. In paper mill medium, the *Burkholderia* biofilm contained more galactose and glucose, and less rhamnose, than in rich laboratory medium. The sugar composition of paper mill slimes was quite similar to those of steel-cultured *B. cepacia* biofilms. This suggests that *B. cepacia* is responsible for much of the slime in the paper mill.

Presently, only a few chemical fine structures of EPS occurring in paper machine slimes are known. An example is levan, which is a fructose-containing polysaccharide. It is synthesized from sucrose by several *Bacillus* and *Pseudomonas* species, which can grow in paper machine recirculated waters. Because this EPS can only be produced in the presence of sucrose, which is in short supply in a paper machine environment, Johnsrud (1997) reports that it is unlikely that this EPS is important for slime formation in paper machines. The dependency of levan production on carbon source availability is an exception on the general assumption that the EPS produced by a microbial species is independent of the carbon source used (Sutherland, 1990) and so it is likely that EPS structures known from other sources could also be present in a paper mill environment. Several Enterobacteriaceae species are known to produce an EPS referred to as colanic acid (Grant et al., 1969). Thirteen strains of *B. cepacia* from various cystic fibrosis clinical isolates, soil, and onion samples were shown to produce EPS with the same structural features. Cerantola et al. (2000) suggested that the two EPSs are representative for *B. cepacia* strains. *Klebsiella* represents a group of slime-forming bacteria having fairly uniform and closely related EPS structures. Their sugar composition mostly consists up to three neutral sugars and an uronic acid, forming repeating units of three to five sugars. The differences between the different EPS structures may be as small as the presence or absence of an acetyl group. O-acetyl and pyruvate ketals are found to be the most common organic

substituents. Bacterial alginate, composed of mannuronic and guluronic acids, is commonly encountered in *Pseudomonas* sp. It is commonly used in laboratory studies on artificial biofilms. This bacterium is commonly isolated from biofilms in the environment, industrial water systems, and infections. Bacterial alginate produced by *P. aeruginosa* is highly O-acetylated and only contains mannuronic acid residues and thus it is different from the alginates isolated from marine algae (Sutherland, 1990, 1994; Davies, 1999).

Because the natural biofilms are composed of mixed species and several different complex EPS structures, enzymes mixtures able to degrade EPS structures need to have different types of highly specific enzymes. The enzymes degrading EPS are commonly not commercially available. The commercial enzymes are hardly found to be capable of degrading these heteropolysaccharides. However, some enzymes are found to be active against bacterial alginates and homopolysaccharides including bacterial cellulose and curdlan. The enzymes are endoglycanases or polysaccharide lyases. The most common sources of these enzymes are microorganisms or bacteriophages. For this reason, most enzymes acting on EPS have to be isolated from the following sources:

- Endogenously from the EPS synthesizing microorganism
- Exogenously from a wide range of other prokaryotic and eukaryotic micro-organisms
- Bacteriophage particles or phage-induced bacterial lysates

Sutherland has published a comprehensive overview of the EPS degrading enzymes (Sutherland, 1995, 1999a,b,c). With respect to enzymatic removal of slime deposits in a paper mill, several approaches have been suggested, resulting in several patents and inventions since the 1970s (Harju-Jeanty, 1988; Hernandez-Mena and Friend, 1993; Carpenter et al., 1990; Aldridge et al., 1993; Hatcher et al., 1973, 1974; Hollis et al., 1992; Wiatr, 1991; van Speybroeck et al., 1996). The first approach is based on preventing the formation of biofilms by enzymatic interference in the process of bacterial adhesion to the surface. The methods are based on the use of protease enzymes alone or in combination with endoglucanases that attack the cell wall of bacteria and therefore prevent them from settling and forming a sessile microcolony (Hollis et al., 1992; Carpenter et al., 1990; Aldridge et al., 1993). The second approach uses lytic enzymes. These enzymes cleave the 1,3-glucose linkages in the bacterial cell wall leading to cell lysis that kills the bacteria (Harju-Jeanty, 1988). The third approach is using a single enzyme—levan hydrolase—attacking only one EPS present in the complex slime layer. This enzyme degrades a fructose containing homopolysaccharide called levan produced by several *Bacillus* sp. (Hatcher, 1973, 1974). Furthermore, the use of a multiactivity enzyme has been found to be effective in the removal of slime layers (van Speybroeck et al., 1996). This enzyme is produced by *Streptomyces* strain capable of degrading colanic acid. Besides its colanic acid-degrading activity, it is also found to have an effect on the biofilms produced by other species such as *Klebsiella*, *Pseudomonas*, and *Xanthomonas*. Other approaches are based upon a mixed enzyme system that contains a range of activities.

Hernandez-Mena and Friend (1993) reported the use of a combination of galactosidase, galacturonidase, rhamnosidase, fucosidase, and α -glucosidase to treat microbial slime within industrial water systems. Also, a patent was filed claiming the use of a combination of β -glucanase, α -amylase, and protease for removing slime (Wiatr, 1991). Moreover, the potential use of the commercial enzyme preparation Pectinex Ultra SP that has a wide range of enzyme activities has been discussed by Johansen et al. (1997). Apart from the use of commercially available enzyme preparations, some studies deal with obtaining enzyme mixtures by culturing bacteria on at least one EPS as a primary carbon source (Rättö et al., 2001). The integrity of complex mixed biofilms is certainly dependent on the presence of different types of macromolecules with polysaccharides and proteins playing a major role. So, the use of a complex mixture of enzymes including both proteases and carbohydrases is more likely to be effective than a single enzyme system. However, none of these methods is adequately well developed to constitute a full-scale alternative to chemical biocides.

An excess of biofilms formed in the machine circuits leads to paper defects or causes web breaks when slime lumps slough off (Alén, 2007; Kolari et al., 2003; Rättö et al., 2005; Väisänen et al., 1998). Several different types of bacteria have been isolated from spots in paper products and deposits in a paper machine. Several bacteria have been found in deposits and paper spots (Ekman et al., 2007; Kolari et al., 2001, 2003; Lahtinen et al., 2006; Denner et al., 2006; Desjardins and Beaulieu, 2003; Busse et al., 2002; Oppong et al., 2003; Väisänen et al., 1998). Ekman et al. (2007) have reported that the most common contaminant is the genus *Meiothermus*, which was found at 18 of 24 machines investigated. Almost one fifth of the deposits contained $\geq 10^9$ *Meiothermus* 16S ribosomal RNA gene copies. Denner et al. (2006) found a novel genus and species—*Rubellimicrobium thermophilum*—from colored paper machine biofilms. The difficulty bacteria has in attaching to clean steel surfaces has been studied by Kolari et al. (2001). They used bacterial strains from the paper industry. *Deinococcus geothermalis* was found to be the primary attacher in the formation of deposits in paper machines. Deposits in the paper industry contain a complex combination of different type of substances. These are fillers, fibers and fines, resins, sizing agents, and binders circulating in the process (Schenker, 1997). Several researchers have commented on the complexity of the deposit formation in the paper industry and the difficulties to draw an exact line between microbial deposits and chemical deposits. The studies show the importance of understanding the mechanisms of their formation (Sanborn, 1965; Alén, 2007). The paper industry spends about 200 million euros annually for so-called slime control (Alén, 2007).

Microorganisms have a sessile and a planktonic phase capable of producing biofilms (Flemming, 2002; Costerton et al., 1987; Ghannoum and O'Toole, 2004). No simple correlation exists between the cell number in the water phase and the biofilm formation. Paper machines with a very high count of colony-forming units in the water circulations are operating without any problems, whereas machines with very low colony-forming units in the water circuits exhibit severe biofilm formations (Alén, 2007; Kanto Öqvist et al., 2001).

3.3 Growth of Micro-Organisms on Raw Materials or Additives

Bacteria colonize all types of wood. Hallaksela et al. (1991) reported that in the winter the major species found in the Norway spruce were spore-forming *Bacillus* sp. During mechanical or semimechanical pulping, some bacterial growth can occur because of the presence of bacteria in the wood and also in the water used for grinding. Thermo-mechanical pulping is done at a temperature of about 80–100 °C, which reduces the amount of micro-organisms present. Carbohydrates are produced during grinding of wood that are used as nutrients by micro-organisms (Biermann, 1996; Lindberg et al., 2004). Chemical pulping is performed at high temperatures (>140 °C), high pH (12), and at high pressure. This process kills all the micro-organisms (Biermann, 1996). All through the papermaking process, conditions support microbiological growth. The major sources of microbiological contamination are fresh water, particularly when surface water without previous treatment is used; the cellulosic raw material, especially when recycled fibers are used; the solutions of additives, starches, fillers, pigments, and coatings; the brokes, particularly when sizing and coating additives are used; and the recycled water and the environment in which the paper machine is placed (Blanco et al., 1997).

Biological contaminants, when they become part of the process water, find an ideal medium for their development. This results in the biofouling phenomena and the deposit of slime (Blanco et al., 1997). When the water circuit is closed, there is an increase in the concentration of nutrients and metabolites and the water temperature and the retention time of the micro-organisms also increases, resulting in an increase in microbial growth. If the concentration of oxygen decreases, the population shifts toward anaerobic species. These are responsible for the problems of odors and corrosion. When the dissolved oxygen concentration is high, aerobic bacteria are developed, which are the major producers of the slime. The population could vary from mesophilic to thermophilic species with the increase of temperature. Under these conditions, spores are produced that are difficult to control (Jung and Kutzner, 1978; Vääänen and Niemelä, 1983; Bennet, 1985; Latorre et al., 1991).

Opportunities for microbiological growth are increased when storage time is long. The tanks, therefore, are often treated with biocides. Additives such as sizing and wet strength agents do not normally contain high loads of bacteria because of preservation by the suppliers. Starches are mostly used as dry strength additive. These are used in the wet-end or in the size press. The consistency of the cooked starch is about 8% dry weight and the consistency of the slurry is about 40% dry weight. Even at high-temperature cooking (130 °C), the retention time at this high temperature is <10 s and allows the heat-resistant spores from the contaminated slurry to grow again in the machine tank (Väisänen et al., 1998; Suominen, 2004). Therefore, biocide treatments are important for reducing contamination of the machine and to prevent the breakdown of the starch molecules that affect the functioning of the starch (Alén, 2007). Treatment of starches with nisin was studied by Pirttijärvi et al. (2001), and the results were encouraging. High microbiological activity in the broke tower has been found to reduce the

redox potential and the pH. This affects the wet end and leads to unstable wet-end conditions that may result in chemical deposition because of unstable process conditions (Alén, 2007). Tanks and vessels, because of little cleaning, may be contaminated with deposits that continuously feed the additives with micro-organisms (Blanco et al., 2004).

3.4 Malodor Formation

When a paper mill closes its water loops, the amount of volatile organic acids increase in the process, which increases the amount of compounds with very strong smells both in the paper and board and also in the surrounding mill. The smell problems are due to the generation of hydrogen sulfide or organic acids produced by bacteria. Hydrogen sulfide production by bacteria is found in paper machine circuits where reductive bleaching agents are used. This problem can be reduced by aerobic and anaerobic microbiological treatment of the water before bringing it back into the process (Blanco et al., 2004; Hamm and Schabel, 2007). The same conditions can also occur in chests and storage towers having long retention time, which promotes anaerobic growth (Alén, 2007). A malodor in finished paper is often related to spore-forming bacteria, both aerobic and anaerobic. The main aerobic bacteria genera in paper industry include *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Gallionella*, *Klebsiella*, *Pseudomonas*, *Sphaerotilus*, and *Thiobacillus*. The most common anaerobic bacteria encountered in the paper industry are *Desulfovibrio* and *Clostridium*. Spores produced by these bacteria are able to survive extreme unfavorable conditions and are mostly very difficult to control. *Bacillus* and *Clostridium* bacterial species are frequently isolated from board samples. These micro-organisms produce high levels of acetic and butyric acid, which produce an unpleasant odor when they germinate in finished products. Normally, an optimized biocide, such as chlorine or hypochlorite in the whitewater system and a conventional biocide in the stock system, together with additives, is required to gain control of spore-forming bacteria. Microbial odor formation is related to the following preconditions (Jung and Kappen, 2010):

- Premicrobial growth with odor-producing micro-organisms
- An oxygen-free (anaerobic) or oxygen-deficient environment
- Sufficient supply of nutrients
- Sufficient retention time
- Factors that support environmental conditions such as specific pH and temperature ranges

Odorous substances are produced microbiologically in the absence of air, whereas emission requires contact with air. Odorous substances pass from the water phase to the air phase at a location other than where they are produced. Once the odorous substances are produced, they are released after transport and a change of environment at locations of high turbulence by stripping or at large boundary layers between water and air. Authorities normally require certifications that the mill is conforming to emissions regulations if the locality around paper mills is affected. Odor inspection can narrow down local areas in which odor emissions are

being released and—as a basis for legal evaluation—the level of the odor pollution is determined in the surrounding areas of the mill. These studies do not provide much information about the sources and causes of odor formation because the odorous substances are simply not produced in the same process step in which they are released. The water circuit should be analyzed for identifying and evaluating the underlying anaerobic microbial metabolic processes. Meaningful inventory can be obtained using the measurands redox potential, strippable hydrogen sulfide, pH, and organic acids. Additional parameters confirm results and help to interpret the results. It is important that the water circuitry and process control be evaluated at the same time the analytical parameters are being determined. Such a process analysis should be carried out by trained personnel to guarantee success. The production of explosive gases hydrogen and methane and bad odors, because of the bacterial action, is important in the mills that use secondary fibers and have a closed water system (Blanco et al, 1997). The growth of methane-producing bacteria and of bacteria of the genus *Clostridium* has been the cause of severe accidents occurred in several board mills (Cox, 1989; Robichaud, 1991; Rowbottom, 1989, 1993; Sorrelle and Belgard, 1991). This type of bacteria may represent a serious problem for the security of the installations if they grow in areas that do not have good ventilation or are stagnant, where the gases could accumulate, reaching higher concentrations than the limit of the explosive threshold. Rowbottom (1989) found that in different board and paper mills where explosions had occurred, they occurred because of hydrogen generation. Moreover, more than 50% of the explosions happened when the paper machine begun to run. Hydrogen could be produced in anaerobic conditions by different genera as *Clostridium*, *Eubacterium*, and *Fusobacterium* (Robichaud, 1991). This problem can be avoided by:

- Preventing the development of the anaerobic bacteria
- Correct ventilation of the chests to reduce the building up of dangerous gases
- The proper use of biocides

The problem of smell in the paper mills is from generation of volatile fatty acids and hydrogen sulfide. The volatile fatty acids are acetic acid, propionic acid, butyric acid, and valeric acid. Several anaerobic micro-organisms produce these acids, and the most common in this industry are those of the genus *Clostridium*, which produces spores and obtains its energy from the metabolism of organic compounds, such as proteins, starch, and other polysaccharides producing acetic, propionic, butyric, and valeric acids. Acetic acid produces a vinegar odor. Butyric acid at a concentration higher than 100 ppm produces a rancid smell to the system that remains in the product. Hydrogen sulfide is produced during the metabolism of anaerobic bacteria, mainly by the sulfate-reducing bacteria. This acid is characterized by a foul odor similar to rotten eggs.

3.5 Quality of the Paper Products

There are several sources from which bacteria enter the papermaking process. They are capable of disrupting the process by slime formation and also can survive in the final paper or board. Paperboard used for food packaging should be free of micro-organisms that can endanger food

safety (Pirttijärvi et al., 1996). Viable bacteria present in food packaging papers mostly consist of bacteria producing heat-stable endospores (Väisänen et al., 1991; Pirttijarvi et al., 1996; Johansson et al., 2001; Suihko and Stackebrandt, 2003) because the vegetative cells are more easily heat-killed in the drying section of the paper machine, where surface temperatures reach up to 140 °C (Väisänen et al., 1998). Densities of bacterial spores in food packaging papers vary from less than 50 up to 105 viable spores/g of paper. *Bacillus* and *Paenibacillus* have been found to be the most commonly found genera (Väisänen et al., 1991; Pirttijarvi et al., 1996; Suominen et al., 1997, 2003; Suihko and Stackebrandt, 2003). *Bacillus cereus* is classified as a pathogen among the bacteria regularly found in the end-products (Anonymous, 2010). The studies by several researchers (Väisänen et al., 1991; Pirttijarvi et al., 1996; Suihko and Stackebrandt, 2003) showed that 5–10% of the viable spores in the end-products represented *B. cereus* sensu lato, which means the genetically highly conserved *B. cereus* group. This group consisted of *B. cereus*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudomycoides*, and *B. anthracis* (Stenfors Arnesen et al., 2008). Quantitative polymerase chain reaction technique was used by Priha et al. (2004) for detecting DNA of the *B. cereus* group bacteria from paper industry end-products with primers described earlier (Hansen and Hendriksen, 2001; Hansen et al., 2001). These researchers found the *B. cereus* group in three of nine samples in amounts corresponding to 102–103 cfu/g of paper (Priha et al., 2004).

Suominen et al. (1997) studied the distribution and growth of bacteria in food packaging paperboards. They used mainly paperboards extruded with polyethene and found that most bacteria were located in the interphase between the polyethene extrusion layer and the web of cellulose fibers. Bacteria enclosed inside the fiber web did not multiply, not even during a 90-day exposure to food and moisture. Bacteria in the interphase of the fiber web and the polyethene layer did multiply, but did not migrate to food under laboratory conditions simulating packaged food. The authors concluded that the main package-related threat to food hygiene was microbially contaminated starches (used as surface sizings) and mineral pigment coatings (Suominen et al., 1997). Johansson et al. (2001) studied the transfer of bacteria from paper to blood agar. They found that during 20 h of contact time (at 4 °C), less than 0.1% of the total bacteria in the paper transferred to the agar surface (Johansson et al., 2001). Some aerobic bacteria—*Bacillus*, *Brevibacillus*, and *Paenibacillus*—are capable of producing heat-resistant spores. In some high-hygiene products such as liquid-packaging board or other food packaging grades, there is a requirement that the amount of spores should be low. Heat-stable endospores will remain viable through the drying section of paper machines. Therefore spore-forming bacteria in the paper machine wet-end can increase the microbial contamination of paper mill final products. This needs attention, especially with machines producing food-quality papers (Harju-Jeanty, 1988; Raaska et al., 2002; Suihko and Hoekstra, 1999; Pirttijärvi et al., 1996; Pirttijärvi, 2000; Sorrelle and Eelgard, 1992; Hughes-van Kregten, 1988; Robichaud, 1991; Väisänen et al., 1991, 1994, 1998; Bennett, 1985; Dyer, 1996; Suihko and Stackebrandt, 2003; Priha et al., 2004). Suominen et al. (1997) have reported that the concentration of bacterial spores can be several times higher at the interface between the polyethylene extrusion and the board, than in the cellulose matrix.

B. cereus has been isolated from slimes in the wire section (Väisänen et al., 1989, 1998), whitewater and calendar water (Pirttijärvi et al., 1999; Pirttijärvi, 2000), and steel coupons immersed in water circuits (Kolari et al., 2001). Suihko et al. (2004) found *B. cereus* from all four mills investigated. They isolated 27 strains of *B. cereus* from broke, slime, water, pulp, and chemical samples and from end-products. Thus, *B. cereus* is a common contaminant in paper machines and can be isolated from many different sites.

Bacterial diversity in the wet-end is typically greater containing mostly gram-negative bacteria than in the end-products, in which the contaminants seem to be limited to the spores that survive the heat of the drying section (Väisänen et al., 1991; Alén, 2007). According to Bendt (1985), the only way to get rid of the endospores is to use oxidizing chemicals; the nonoxidizing biocide glutaraldehyde is also considered to be efficient against endospores.

3.6 Microbially Influenced Corrosion

Microbially influenced corrosion imposes industry with major economic problems because of its effects on the machinery (Blanco et al., 1996). The corrosion induced by micro-organisms is directly or indirectly produced as a result of their metabolic activity and is generally associated with the microbiological deposits. Biofilms are known to mediate interactions between metal surface and the environment. Depending upon environmental conditions and available nutrients, the biofouling film may comprise a wide range of aerobic, facultative, and anaerobic micro-organisms that together hydrolyze and ferment carbohydrates, proteins, and many other primary nutrients. Consumption of oxygen by the aerobes results in the formation of anaerobic pockets and the growth of sulfate-reducing bacteria that accelerate localized corrosion of surfaces. Corrosion can occur below biofilms on the machine surfaces. Uneven biofilm coverage creates neighboring liquid cells with different redox potentials, oxygen, and chloride concentrations on the metal surface; this is a driving force for the corrosion. Other microbiological products like acids and hydrogen sulfides further speed up corrosion (Alén, 2007; Blanco et al., 1996; Heitz et al., 1996).

In the recent past, several cases of engineering failure because of biofilm accumulation have been reported. There is an increasing recognition of the problems caused by microbial fouling. The failures attributed to these biofilms are:

- Impairment of heat-transfer efficiency
- Increased frictional resistance
- Clogging of valves and filters
- Accelerated localized corrosion

Although the conventional antifouling paint prevents the settlement of macrofouling, it does not offer protection against the biofilms comprising bacteria, diatoms, and protozoans. Recent restrictions on the use of chromate-based inhibitors and the use of biocides have resulted in

extensive growth of microbial films on the immersed surfaces. A considerable body of information exists on the development of biofilms on various metallic materials under different environmental conditions. One of the most important implications of biofilm formation is the accelerated corrosion of metallic surfaces. Biofilm formation, which involves micro-organisms, their metabolic products, exopolymers, trapped detritus, and the other organic matter, changes the physical and chemical nature of the metal surface. Thus, microbial film considerably affects the kinetics of corrosion and also the type of corrosion the metal suffers. Different types of bacteria colonizing biofilm cause corrosion by various mechanisms—varying from formation of differential aeration cells to production of aggressive environments through chemical changes. Consumption of oxygen by the aerobes in biofilm results in oxygen-depleted conditions favoring the growth of sulfate-reducing bacteria, substantially accelerating localized corrosion of surfaces and causing severe maintenance problems. Metals like aluminium and its alloys, stainless steels, and copper alloys having a stable oxide film for their corrosion resistance are vulnerable to this form of corrosion because of the damage in oxide film or oxygen depletion on the metal surface by the biofilm. Corrosion can also be accelerated by the chemistry which develops within the biofilm and the exopolymers produced with metal-binding abilities. Main metabolic products that cause the microbiologically induced corrosion are presented in Table 3.9 (Väätänen and Niemelä, 1983; Randrup, 1994). The corrosion can be produced by different types of micro-organisms, including bacteria, fungi, and algae and are generally associated with microbiological deposits. Sulfur oxidizers, mainly of the genus *Thiobacillus*, form sulfuric acid, which is a strongly corrosive agent. The aerobic iron bacteria in the genera *Gallionella*, *Pedomicrobium*, and *Siderococcus* oxidize the ferrous iron into ferric iron, catalyzing the deposition of tubercles (Herro, 1991). Members of the filamentous bacteria of the genus *Leptothrix* deposit ferric oxides in their sheathes. Some species of *Metallogenium* oxidize the manganese originating the deposits of salts of Mn^{4+} out of the cellular wall.

Table 3.9: Metabolic products causing microbiologically induced corrosion

Metabolic product	Microorganisms	Corroded material	Effects
Ammonia	NO_2 reducing bacteria	Copper and alloys	Formation of soluble complexes
Hydrogen	Hydrogen producers	Tanks with dead zones	Explosion risk
Methane	Methanogenic bacteria	Tanks with dead zones	Explosion risk
Carbon dioxide	Fermentatives	Iron	Carbonic acidity
H^+	Organic acids producers	Iron and alloys, except stainless	Acidity
Sulfuric acid	<i>Thiobacillus</i>	Most of metals	High acidity
Fe^+	Iron oxidizers	Iron and alloys	Redox abiotic
Hydrogen sulfide	Sulfate reducing bacteria	Metals or alloys, even stainless	Acidity, insoluble salts

Based on Blanco et al. (1997).

Microorganisms can influence corrosion in several ways. Formation of localized differential cells and the production of mineral and organic acids, ammonia production, and sulfate reduction are just a few of the mechanisms by which bacteria, fungi, and algae can affect corrosion. The primary mechanism of corrosion caused by iron-oxidizing bacteria, such as *Gallionella* sp., *Arthrobacter siderocapsulatus*, and *Pseudomonas putida*, is the formation of localized cells. When iron- and manganese-oxidizing bacteria colonize a surface, they start to oxidize available reduced forms of these elements. This results in the production of deposits. In the case of iron-oxidizing bacteria, ferrous iron is oxidized to ferric ion, with the electron lost in the process, which is used by the bacterium for energy production. As the bacterial colony gets encrusted with iron (or manganese) oxide, a differential oxygen concentration cell may develop, and the corrosion process starts to begin. The ferrous iron that is generated at the anode will then provide more ferrous iron for the bacteria to oxidize. The porous encrustation may potentially become an autocatalytic corrosion cell or may provide appropriate environment for growth of sulfate-reducing bacteria. Growth of iron-oxidizing bacteria results in localized corrosion cell development. Figures 3.2, 3.3 and 3.4 illustrate the stages. Corrosion can also develop when localized cells are produced, simply because of biofilms developing on metal surfaces. The oxidation of iron or manganese is not a prerequisite for the development of a localized corrosion cell.

Various factors can contribute to localized corrosion on metal surfaces. The generation of ammonia by the reduction of nitrates or nitrites may lead to serious localized loss on copper-based metallurgy. The production of organic acids, such as acetic, butyric, or citric acid, may help in the solubilization of protective metal oxide films. Inorganic acid, such as sulfuric acid produced by *Thiobacillus* sp., can also have harmful effects. As the biofilms grow, they will ultimately achieve a thickness at which oxygen concentration is either very low or completely excluded. The oxygen concentration within the biofilm is reduced to near zero ppm at a

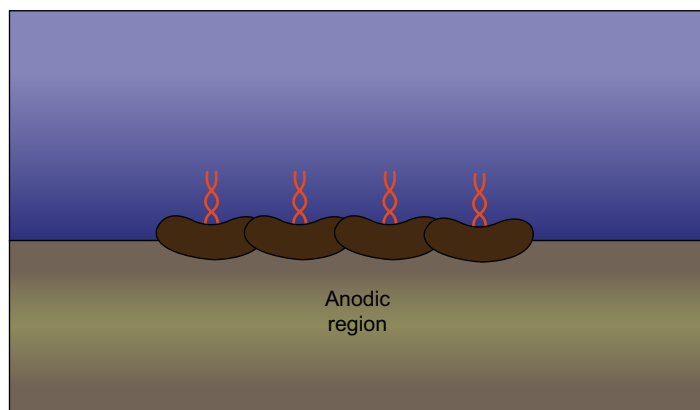


Figure 3.2

Bacterial growth and iron oxidation. From Buckman performance chemicals: Biofilms copyright 2010 by Buckman laboratories international Inc. Reprinted with permission from Buckman laboratories international Inc.

thickness of just 200 μm . When this happens, facultative and obligate anaerobes (such as *Desulfovibrio* sp. and *Clostridium* sp.) can thrive. Anaerobic sulfate-reducing bacteria (e.g., *Desulfovibrio*) are the bacteria causing microbial corrosion. These organisms can grow in areas lacking oxygen, like those found within porous corrosion tubercles, within biofilms and under debris. These bacteria cause fast and rigorous metal loss in industrial water systems. This type of corrosion can be easily identifiable from the characteristic sulfide byproduct present within the corrosion cell. Sulfate-reducing bacteria basically cause corrosion by using the molecular hydrogen generated at the cathode, and depolarizing it. Removal of cathodic reduction products will increase the rate of corrosion as the rate of corrosion is under cathodic control. Systems that are sulfate-limited will have lesser tendency to be attacked by sulfate-reducing bacteria.

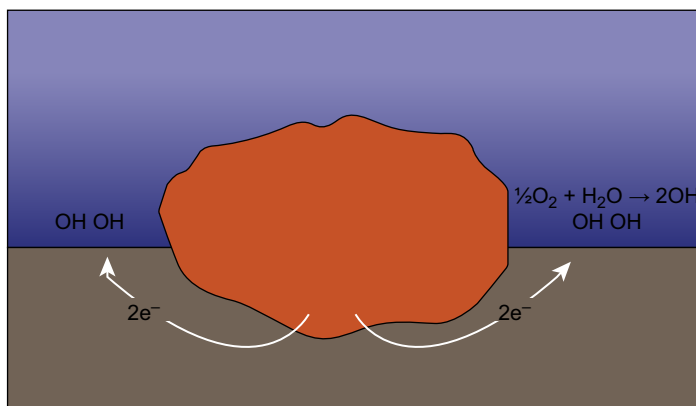


Figure 3.3

Corrosion cell development. From Buckman performance chemicals: Biofilms copyright 2010 by Buckman laboratories international Inc. Reprinted with permission from Buckman laboratories international Inc.

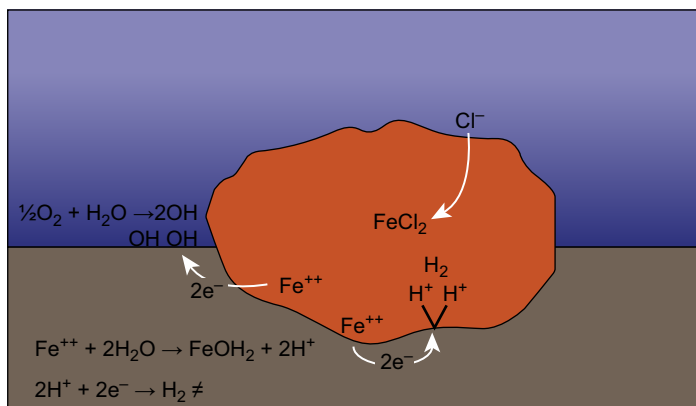


Figure 3.4

Tubercle and autocatalytic cell development. From Buckman performance chemicals: Biofilms copyright 2010 by Buckman laboratories international Inc. Reprinted with permission from Buckman laboratories international Inc.

Stainless steels are extensively used as construction materials in the pulp and paper industry. The whitewaters of paper and board machines are normally considered benign and quite harmless to stainless steels especially when the chloride contents are low. However, if the conditions change substantially (e.g., from microbial activity or increased concentrations of harmful ions or increased temperature from the closure), localized corrosion of stainless steels in the paper machine environment is possible. Often these failures have occurred at splash zone areas at the wet-end of paper machines. The conditions for microbial growth are extremely favorable in the papermaking process, as mentioned previously. There are both organic and inorganic nutrients in whitewaters and the pH value as well as the temperature is at the appropriate level for the microbes to survive and multiply. Chlorides, sulfates, thiosulfates, and other sulfur compounds and the molar ratios in which they exist are essential for considering the risk of stainless steel corrosion. Traces of thiosulfate in otherwise harmless whitewaters can cause heavy pitting in austenitic stainless steel UNS S30400 (AISI 304). Even though more resistant to the effects of thiosulfate, corrosion of stainless steel type UNS S31600 has occurred in a paper machine wet-end. Thiosulfate appears as a breakdown product of sodium hydrosulfite and dithionite. Thiosulfate can also be present as an oxidation product of hydrogen sulfide produced by anaerobic bacteria. Sulfate-reducing bacteria, like *Desulfovibrio* and *Desulfotomaculum*, are anaerobes and normally require a complete absence of oxygen and a highly reduced environment. A redox potential around $-100\text{ mVsHz} = -342\text{ mVscz}$ is needed for sulfate-reducing bacteria to thrive.

The biofilm formation and electrochemical behavior of stainless steels were studied by Carpen et al. (2001) in environments simulating the splash zone areas of paper machines. A novel glass dome arrangement for laboratory tests was used by these researchers. As a test environment, simulated whitewater using mixed communities with sulfate-reducing bacteria and aerobic bacteria was applied. The potentials were recorded and anodic polarization curves were determined. The biofilms formed were analyzed by microbial cultivation and microscopic examination and biofilm population was characterized using in situ hybridization. The sulfur compounds, oxalate as well as chloride content, in the simulated whitewater were analyzed by capillary electrophoresis. The biofilm formation was clearly more representative than in earlier studies performed with totally immersed specimens in modified Robbins device. Also the biofilm formed resembled that formed at the field in real mill conditions. Using this test arrangement, it was possible to add both aerobic and anaerobic bacteria simultaneously. Pitting initiated easily on the UNS S30400 stainless steel, when the amounts of both aerobic bacteria and sulfate-reducing bacteria were high after the test. However, the high amount alone was not enough; the bacteria had to be active as well.

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Factors Affecting Biofilm Development

Over the past few decades, biofilm growth has been observed in many industrial and domestic domains. Bacterial biofilms are considered to be the predominant mode of life of bacteria in nature. These are multicellular aggregates of cells attached to surfaces or interfaces and are bound together by an extracellular matrix <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0078524> - pone.0078524-Brandal (Flemming and Wingender, 2001; Stoodley et al., 2002a,b; O'Toole et al., 2000). This matrix is composed of extracellular polymeric substances (EPS) such as proteins, nucleic acids, amyloid fibrils, and other components produced by bacterial cells. Biofilm formation is often triggered by changes in the environment and involves several stages often referred to as the biofilm lifecycle. These steps include attachment of the floating cells to a surface, maturation, maintenance, and dissolution. Multicellular living offers many advantages for the constituent cells. A major benefit is protection by the surrounding matrix from environmental stresses such as pH shifts, desiccation, ultraviolet radiation, and osmotic shock (Davies et al., 1998). Bacterial biofilms present a major issue in many industrial, environmental, and medical applications. Several articles have been published on genetics, biochemistry, and biology of biofilms but there are not many studies concerning the physical and mechanical properties of biofilms (Aggarwal et al., 2010; Ahimou et al., 2007; Kamjumphol et al., 2013). Moreover, not much information is available about the change of the properties during the course of biofilm development and how this is affected by different environmental parameters.

The growth of biofilms has been detrimental in most cases. Many industries suffer the adverse effects of biofilm growth of one type or another. This can result in substantial costs in cleaning and maintenance. Examples of such industries include the paper, dairy, food, water systems, oil, opticians, dentistry, and hospitals. Perhaps the environment where people are exposed to biofilms most frequently is the domestic one (Busscher and Van Der, 1995; Yoo and Chen, 2002; Verran and Jones, 2000; Ganesh and Anand, 1998; Bott, 1998; Nemati et al., 2001; Klahre and Flemming, 2000; Liesegang, 1997 Marotta et al., 2002; Halabi et al., 2001; Barker and Bloomfield, 2000; Scott et al., 1982).

A few examples of the detrimental effects of biofilms are:

- Product spoilage
- Reduced production efficiency
- Corrosion
- Unpleasant odors (malodors)

- Unsightliness
- Infection
- Pipe blockages
- Equipment failure

Because of these reasons and the emergence of restrictive legislation regarding the effects of cleaning agents on the environment and to user health and safety (Commission Regulation EC No. 1048/2005), a lot of industrial interest is being shown in developing materials and methods that can remove and actively prevent the formation of biofilms.

The development of biofilm at a surface is the net result of several physical, chemical, and microbial processes, as mentioned in Chapter 3. Several factors affect biofilm development (Garrett et al., 2008; Kim et al., 2012; Goller and Romeo, 2008), including temperature, pH, O₂ levels, hydrodynamics, osmolarity, and the presence of specific ions, nutrients, and factors derived from the biotic environment. The combination of these effects eventually determines the pattern of behavior of a given bacterium with respect to biofilm development.

4.1 Nutrients

Biofilms are able to form under different nutrient concentrations, ranging from high to almost nondetectable. In environments with high nutrient levels, they are found to be more abundant and thicker (Allison et al., 2000; Rochex and Lebeault, 2007). High nutrient concentrations actually promote the transition of bacterial cells from the planktonic form to biofilm state, whereas exhaustion of these nutrients is found to cause detachment of biofilm cells from surfaces (O'Toole et al., 2000; Allison, 1998; Hunt et al., 2004; Rochex and Lebeault, 2007). In an open reticulating system, there are plentiful nutrients originating from water, particularly in cooling towers. Closed systems (not exposed to the atmosphere) are found to be perfect systems in that the biofouling problem is either unlikely to be encountered or significantly reduced (Melo and Bott, 1997; Melo and Vieira, 1999). High concentrations of nutrients appear to produce an open structure in the biofilm, whereas lower levels tend to produce a more compact structure. The structure of the biofilm has an effect on the availability of nutrients to the constituent cells. An open structure facilitates the diffusion of nutrients to the bacteria (Allison, 2003). The availability of oxygen is necessary for aerobic bacteria, unless the particular microorganism can exist under oxygen-starved conditions (Melo and Bott, 1997). An increase in nutrient concentrations correlates with an increase in the number of attached bacterial cells. However, low nutrient concentrations are still sufficient for biofilm growth. Cowan et al. (1999) have reported that biofilm bacteria acquire nutrients by:

- Concentrating trace organics on surfaces by the extracellular polymer using the waste products from previously attached cells and secondary colonizers
- By pooling the biochemical resources with different enzymes to break down food supplies

4.2 Effect of Temperature

The optimum temperature for a microorganism is linked with an increase in nutrient intake (Garrett et al., 2008). This results in a rapid formation of biofilm (Stepanovic et al., 2003). Metabolism of nutrients is directly linked and is dependent on the presence of enzymes. So the formation of a biofilm depends on the presence and reaction rates of enzymes, which actually controls the development of many physiological and biochemical systems of bacteria. Temperature is correlated with the reaction rate of enzymes and therefore has an effect on the development of the cells. Optimum temperatures result in the healthy growth of bacterial populations. Conversely, temperatures away from the optimum results in reduced bacterial growth efficiency because there is a reduction in bacterial enzyme reaction rates. Environmental temperature affects the physical properties of the compounds within and surrounding the cells in addition to enzymes.

For many bacteria, the optimum temperature for maximum growth is about 40 °C (Melo and Bott, 1997; Timothy and Hansen, 2006). At this temperature, small changes in temperature are likely to produce substantial changes in the growth of biofilms (Dewanti and Wong, 1995), because microbial activity is very sensitive to temperature. For instance, studies have shown that biofilm thickness of *Escherichia coli* increased by 80% by increasing the temperature from 30 °C to 35 °C (Melo and Bott, 1997). In the case of *Stenotrophomonas maltophilia* isolates, more formation of biofilm was observed at 32 °C than at 37 °C or 18 °C (Di Bonaventura et al., 2007). Low temperatures (20 °C) stimulated more biofilm formation in case of *Burkholderia pseudomallei* than 30 °C or 37 °C. On the other hand, protease production and bacterial motility were adversely affected but not in the case of low temperature (Kamjumphol et al., 2013).

Fletcher (1977) reported the effect of temperature on attachment of stationary phase cells. Results showed that a decrease in temperature reduced the adhesive properties of a marine pseudomonad. It is believed that the effect was due to a decrease in the bacterial surface polymer at lower temperatures and other effects such as a reduced surface area. However, Herald and Zottola (1988) observed that the presence of bacterial surface appendages was dependent on the temperature. At 35 °C, cells were shown to have a single flagellum, whereas at 21 °C they had two to three flagella and at 10 °C, cells exhibited several flagella. This suggests that the initial interaction between the bacteria and substrate may increase with a lowering of temperature, increasing the possibility of adhesion. Possibly, the more uniform properties of polysaccharides at lower temperatures increase the possibility of biofilm adhesion, because many microbial polysaccharides undergo transition from an ordered state at lower temperatures and in the presence of ions to a disordered state at high temperature under low ionic environments (Nisbet et al., 1984). Although much information is available in the literature describing the effect of temperature on bacterial growth in culture, not much is reported on the effect of temperature on removal of adhered microorganisms. Marion-Ferey et al. (2002) observed the effect of high temperatures

(80–90 °C) on the removal of biofilms. They found that these temperatures were not effective for biofilm removal due to “baking effects” at high temperature, which increased the adherent nature of the biofilm to the surface.

4.3 Effect of pH

Changes in pH can have a profound effect on bacterial growth. Bacteria possess membrane-bound proton pumps that extrude protons from the cytoplasm to produce a transmembrane electrochemical gradient (i.e., the proton motor force) (Rowland, 2003). The passive influx of protons in response to the proton motive force can be a problem for cells attempting to regulate their cytoplasmic pH (Booth, 1985). Large differences in external pH can overcome such mechanisms and have a biocidal effect on the microorganisms. Olsen (1993) reports that bacteria respond to changes in internal and external pH by adjusting the activity and synthesis of proteins associated with many different cellular processes. Li (2001) has shown that a steady increase in acidity increases the possibility of cell survival in comparison to a sudden increase by rapid addition of hydrochloric acid. This implies that bacteria contain mechanisms in place that allow the bacterial population to adapt to small environmental changes in pH. But, there are cellular processes that do not become accustomed to pH variations so easily. One such process is the excretion of exopolymeric substances (polysaccharides). Optimum pH for polysaccharide production depends on the individual species, but for most bacteria, it is around pH 7.0 (Oliveira et al., 1994). Di Bonaventura et al. (2007) reported that in case of *Stenotrophomonas maltophilia* isolates, biofilm production at pH 7.5 and 8.5 was comparable but significantly higher than at pH 5.5.

4.4 Surface Condition

The attachment of microorganisms to surfaces is a complex process. Many variables are found to affect the results. Surfaces that are rougher, more hydrophobic, and coated by surface conditioning films are found to promote attachment of microorganisms most readily (Zacheus et al., 2000; Dunne, 2002). In addition, growth requires several complex developmental pathways. These pathways are regulated in response to environmental and bacterial derived signals. Prakash et al. (2003) conducted studies on the effect of substratum and found that the extent of microbial colonization increases as the surface roughness increases (Prakash et al., 2003). Studies have shown that the surface condition whether rough or smooth affects the ability of bacteria to adhere to a surface. These researchers showed that a material surface exposed in an aqueous medium will unavoidably become conditioned or coated by polymers from that medium; the resulting chemical modification will affect the rate and the extent of microbial attachment.

The surface may have several properties that are important in the attachment process (Sauer and Camper, 2001). An increase in flow velocity, water temperature, or nutrient concentration may also promote increased attachment provided these factors do not exceed critical levels (Donlan

and Costerton, 2002). Properties of the cell surface, particularly the presence of fimbriae, flagella, and surface-associated polysaccharides or proteins, are also found to be very important and may provide an advantage for one microorganism where a mixed culture is involved (Zobell, 1943; Donlan and Costerton, 2002; Donlan, 2002). Surfaces cannot be colonized by biofilms unless they have been exposed to organic material from the surrounding environment (Allison et al., 2000). But, the effect of several surface characteristics—charge, hydrophobicity, roughness, and elasticity on microbial attachment—cannot be overlooked (Allison et al., 2000).

4.5 Flow Velocity and Hydrodynamics

The flow velocity has been shown to be insufficient for biofilm removal within the boundary layer (Dreeszen, 2003). The boundary layer is the area from the surface where no turbulent flow is experienced. The area outside the boundary layer is characterized by high levels of turbulent flow and affects the attachment of cells to the surface (Donlan and Costerton, 2002; Donlan, 2002). Studies showed that an increase in water flow velocity resulted in an increase in bacterial number in biofilms. This can be attributed to better mass transfer of growth limiting nutrients at the higher flow velocity of water (Vieira et al., 1993; Lehtola et al., 2006). Biofilms depend on defensive mechanisms to resist detachment by the higher fluid shear. Viscoelasticity of biofilms allows them to resist detachment as has been reported in case of *Staphylococcus aureus* biofilms (Lehtola et al., 2006). The size of the boundary layer is dependent on the flow velocity of the water. At high velocities, the boundary layer decreases in size and the cells are exposed to high turbulence levels (Donlan and Costerton, 2002; Donlan, 2002).

Hydrodynamic conditions can affect the metabolic activities of biofilms. Also, the formation, structure, EPS production, thickness, and mass are affected (Stoodley et al., 2002a,b; Liu and Tay, 2002a,b; Simoes et al., 2003). Studies regarding the hydrodynamic of aqueous medium showed that the flow velocity adjacent to the substratum/liquid interface is negligible. This zone of negligible flow is termed as the hydrodynamic boundary layer (Kumar and Prasad, 2006). Its thickness is dependent on linear velocity. The higher the velocity, the thinner the boundary layer. The region outside the boundary layer is characterized by vigorous mixing or turbulence. The hydrodynamic boundary layer may affect cell substratum interactions for flow regimes characterized as laminar or minimally turbulent (Kumar and Prasad, 2006). Stoodley et al. (2002b) have reported that biofilms formed under low-shear conditions (laminar flow conditions) are characterized by spherical microcolonies divided by water channels. Simoes et al. (2010) studied the differences between three strains of *Pseudomonas fluorescens* biofilms that were grown under turbulent and laminar flow. All biofilms grown under turbulent flow were found to be denser, had a higher mass, were more active, and produced similar amounts of matrix proteins; the *P. fluorescens* strains had higher amounts of extracellular polysaccharides. Several studies have shown that the biofilms formed under higher detachment forces produced more extracellular polysaccharides to stabilize the biofilm structure and to withstand the shear force (Ohashi and Harada, 2004; Chen et al., 1998)

4.6 Rheological and Adhesive Properties of Biofilms

Pure culture biofilms and mixed species act like viscoelastic fluids. Biofilms show irreversible viscous deformation and reversible elastic response and recoil (Ohashi and Harada, 2004). Extracellular polymeric substances such as alginate, xanthan, and gellan gum aggregate because of hydrogen bonding to form highly hydrated viscoelastic gels (Stoodley et al., 1999a,b). The presence of acetylated uronic acids in the bacterial alginate of *Pseudomonas aeruginosa* biofilms increases its hydration capacity (Ganesh and Anand, 1998). These properties provide the biofilm with mechanical stability (Stoodley et al., 2002a,b). The matrix formed by EPS responds to stress by showing (Klapper et al., 2002):

- (1) Elastic tension from a combination of polymeric entanglement, entropic, and weak hydrogen bonding forces
- (2) Viscous damping from polymeric friction and hydrogen bond breakage
- (3) Alignment of the polymers in the shear direction

These properties change with increased temperature. Increasing the temperature of polysaccharides produces a gel-like substance that steadily increases in strength until a critical point is reached. At this point, the gel forms a solution (Villain-Simonnet et al., 2000). This type of behavior affects the viscosity of the polysaccharides, which may affect biofilm adherence. Rühls et al. (2013) presented a novel method to measure biofilm strength: interfacial rheology. By culturing a range of bacterial biofilms on an air-liquid interface, they were able to measure their viscoelastic growth profile during and after biofilm formation and subsequently alter growth conditions by adding surfactants or changing the nutrient composition of the growth medium. They found that different bacterial species had unique viscoelastic growth profiles, which was also highly dependent on the growth media used. They could reduce biofilm formation by adding surfactants or changing the pH, thereby altering the viscoelastic properties of the biofilm. Using this technique, they were able to observe changes in viscosity, elasticity, and surface tension online, under constant and varying environmental conditions, and thus provide a complementary method to better understand the dynamics of both formation and dispersal of biofilm.

4.7 Effects of Particles

Biofouling of industrial equipment crop up together with other kinds of fouling. The most common being the concurrent deposition of small particles that are transported with the incoming water and/or those formed in the plant as a result of metal corrosion (Vieira et al., 1993). When the particles are organic in nature, they act as substrate for microorganisms and are degraded by them contributing to the growth of the biomass (Melo and Bott, 1997). In most cases, the biological matrix incorporates inorganic particles that are relatively inert but may cause changes in the structure and activity of the biofilms (Battin et al., 2003a,b). The adhesion between particles and microorganisms could be facilitated by the electropositive charges. These charges are developed on the surfaces of some particles depending on the pH

of the environment. Toxic metallic ions and metabolic inhibitors could be adsorbed on the particle surface supporting the formation of biomass and stimulation of microbial respiration in the presence of particles (Melo and Bott, 1997).

4.8 Properties of the Cells

The hydrophobicity of the cell surface, the presence of fimbriae and flagella, and production of EPS affect the rate and extent of attachment of microbial cells (Liu and Tay, 2002a,b). The hydrophobicity of the cell surface is very important in adhesion. This is because hydrophobic interactions tend to increase with an increasing non polar nature of one or both surfaces—the microbial cell surface and the substratum surface—involved. Most bacteria are negatively charged but still contain hydrophobic surface components (An et al., 2000). Fimbriae contribute to cell surface hydrophobicity. Most fimbriae that have been studied contained a high percentage of hydrophobic amino acid residues. Fimbriae play a role in cell surface hydrophobicity and attachment, most likely by overcoming the initial electrostatic repulsion barrier that is found between the cell and substratum. Spiers et al. (2003) have reported that several aquatic bacteria possess fimbriae that are involved in bacterial attachment to animal cells. Treatment of adsorbed cells with proteolytic enzymes caused a significant release of attached bacteria, providing evidence for the role of protein in attachment (Donlan and Costerton, 2002; Donlan, 2002). Spiers et al. (2003) found that mycolic acid containing organisms such as *Corynebacterium*, *Nocardia*, and *Mycobacterium* were more hydrophobic than the non-mycolic acid containing bacteria. The increase in the chain length of mycolic acid coincided with an increase in hydrophobicity. Adhesion was found to be greater on hydrophobic materials for most of the bacterial strains studied. A study by Spiers et al. (2003) reported that the O antigen component of lipopolysaccharide imparts hydrophilic properties to gram-negative bacteria.

4.9 Gene Regulation

Studies based on gene regulation of microbial biofilms were made by Steyn et al. (2001). It was found that 22% of the genes were upregulated and 16% downregulated in biofilm-forming bacteria *P. aeruginosa*. In another study, AlgC was upregulated within minutes of attachment to a surface in a flow cell system. Genes encoding for enzymes involved in glycolysis or fermentation (triosephosphate isomerase, phosphoglycerate mutase, and alcohol dehydrogenase) were up regulated in biofilm formation of *S. aureus*. Study by Prakash et al. (2003) showed that AlgD, AlgU, RpoS, and genes controlling polyphosphokinase synthesis were upregulated in biofilm formation of *P. aeruginosa*.

4.10 Quorum Sensing

Bacteria use quorum sensing to coordinate certain behaviors such as biofilm formation, virulence, and antibiotic resistance based on the local density of the bacterial population.

Quorum sensing can occur within a single bacterial species and also between the diverse species, and is able to regulate a host of different processes serving as a simple indicator of population density or the diffusion rate of the cell's immediate environment (Hammer and Bassler, 2003; Shrout et al., 2006; Xiong and Liu, 2010). Quorum sensing is dependent on the cell density. The development of biofilms on surfaces is mediated by a density-dependent chemical signal produced by bacterial cells densely packed within an EPS matrix. According to de Kievit et al. (2001), quorum systems make use of a transcriptional activator protein that acts in concert with a small autoinducers signaling molecule to stimulate expression of target genes. Increasing bacterial density gives rise to an accumulation of autoinducers (Xiong and Liu, 2010). Once the critical concentrations of autoinducers are attained, the regulator of proteins is activated and further induces target DNA sequence. This leads to transcription of quorum-sensing regulated genes and will then result in changes of bacterial behavior (Decho, 2000; Xiong and Liu, 2010). Fuqua et al. (1996) and Dunlap (1997) report that this type of intercellular communication serves to coordinate gene expression and structures morphological differentiation and development responses of bacterial cells.

Quorum-sensing signals are also able to control detachment of biofilm by the accumulation of the signal molecules produced by bacteria to a threshold concentration that will ultimately activate the dispersion of the biofilm (Hentzer et al., 2002). Study with *P. aeruginosa* biofilms in continuous-flow conditions showed that these biofilms detached after flow was stopped. When these biofilms were starved from nutrients under continuous-flow conditions, detachment also occurred. These observations suggest that starvation and not the accumulation of signal/metabolic products was responsible for triggering the detachment of these biofilms (Hunt et al., 2004; Nadell et al., 2008).

Cell-to-cell communication is essential for biofilm formation and is closely regulated to autoinducers. There are three types of autoinducers that have been identified (Davies et al., 1998) (Pierson et al., 1998; Xiong and Liu, 2010).

- Oligopeptides
- *N*-acyl homoserine lactones (ahl)
- Autoinducers-2 (AI-2) synthesized by LuxS

Oligopeptides and AHL are involved in cellular communication of gram-positive and gram-negative bacteria respectively, whereas AI-2 played a role in the interspecies communication of both gram-positive and gram-negative bacteria. Autoinducers mediated quorum-sensing systems play a very important role in biofilm formation and the regulation of microbial attachment (Xiong and Liu, 2010).

Kim et al. (2012) have discussed bacterial biofilm development as a structurally and dynamically complex biological system and propose microfluidic approaches for the study of bacterial biofilms. Table 4.1 summarizes the effects of the environmental conditions, including the surface properties, hydrodynamic conditions, quorum-sensing signals, and characteristics of the medium.

Table 4.1: Effects of the environmental conditions on biofilm development

Effect on biofilms		Microorganism
Surface properties^a		
Surface roughness	Positive	<i>Pseudomonas aeruginosa</i>
Hydrophobicity	Positive	<i>Pseudomonas</i> sp.
Nonpolar surface	Positive	<i>Pseudomonas</i> sp.
Porosity	Positive	<i>Corynebacterium</i> , <i>Rhodococcus</i> , <i>Gordona</i>
Cations on the surface	Positive	<i>Pseudomonas fluorescens</i>
Chloropropyl-terminated surface	Positive	<i>P. fluorescens</i>
Alkyl-terminated surface	Negative	<i>P. fluorescens</i>
Nanostructure of the surface	Positive/negative	<i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i>
Characteristics of the aqueous medium^b		
Nutrient source	Positive/Negative	<i>P. aeruginosa</i>
Nutrient starvation	Negative	<i>P. aeruginosa</i>
Oxygen concentration in the fluid	Positive	<i>P. aeruginosa</i>
Carbon dioxide concentration in the fluid	Positive	<i>Pseudomonas putida</i>
Dense phase carbon dioxide	Negative	<i>P. aeruginosa</i>
Quorum-sensing signals^c		
Quorum sensing autoinducers	Positive	Gram-negative bacteria
Hydrodynamic conditions^d		
Residence time	Positive	<i>P. aeruginosa</i>
Shear stress at the interface	Positive/negative	<i>P. aeruginosa</i> , <i>P. fluorescens</i>
Hetero-stress distribution at the interface	Negative	<i>P. aeruginosa</i>

^aBased on Puckett et al. (2010), Flemming, and Wingender (2001), Kinnari et al. (2009), Kapellos et al. (2007), Song and Leff (2006), Brizzolara and Holm (2006).

^bBased on Skolimowski et al. (2010), Shrout et al. (2006), Hunt et al. (2004), Applegate and Bryers (1991), Mun et al. (2009).

^cBased on Miller and Bassler (2001).

^dBased on Lecuyer et al. (2011), Ochoa et al. (2007), Salek et al. (2009), Tsai (2005).

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Microorganisms Occurring in Papermaking Process and Their Location

5.1 Microorganisms Occurring in Papermaking Process

The types of microorganisms encountered in the papermaking process may be diverse and can include aerobic spore-forming bacteria (*Bacillus*), aerobic nonsporulating bacteria (e.g., *Acinetobacter*, *Alcaligenes*, *Klebsiella*, *Flavobacterium*, *Leptothrix*, *Micrococcus*, *Pseudomonas*, and *Staphylococcus*), anaerobic bacteria (e.g., *Desulfovibrio*), molds (e.g., *Aspergillus*), yeasts, and occasionally also algae (Table 5.1) (Lahtinen et al., 2006; Desjardins and Beaulieu, 2003; Väisänen et al., 1989, 1991, 1994, 1998; Kolari et al., 2001, 2002, Kolari, 2003, 2007; Oppong et al., 2000, 2003; Ekman et al., 2007; Ekman, 2011; Denner Ewald et al., 2006; Busse et al., 2002; Stanier et al., 1968; Sanborn, 1965; Lutey, 1972; Eveleigh and Brewer, 1963; Brewer, 1960; Harju-Jeanty and Väättänen, 1984; Martin, 1955; Safade, 1988; Latorre, 1991; Lindberg, 2001a,b; Prince et al., 2009; Kashama et al., 2009; Suihko and Skytta, 2009; Suihko et al., 2005).

The most common of the slime-producing bacteria belonging to this group is *Aerobacter aerogenes*. This bacterium is a nonsporing rod, having a large capacity for adapting to the oxygen supply of the environment. It is very often found in paper mills and gives rise to a soft and gelatinous slime, which requires the mechanical support of a network of paper fibers. Its multiplication is particularly large in mill waters, which have a very high biochemical oxygen demand as a result of the substantial concentration of organic materials. Several variants of this species have been found, and they are mobile and can select the most appropriate location of the paper machine for forming colonies. Other bacterial species detected in slime are *Chlamydobacteriales* (encapsulated bacteria), *Alcaligenes*, *Arthrobacter*, *Proteus*, *Bacillus*, *Escherichia coli*, pseudomonads, and others. *Escherichia coli* is basically a microorganism of the intestinal flora, which resembles *A. aerogenes* in several respects but produces a film of slime that gradually increases in thickness with time and finally tears off in the form of small grayish scales. Its nutrient requirements are similar to those of *Aerobacter*. Pseudomonads comprise a range of badly defined species and make a special contribution to slime formation. These are found to be extremely resistant to low concentrations of antislime agents. *Chlamydobacteriales* require higher level of oxygen in contrast with the *A. aerogenes* and *E. coli*, and is an important group of slime-generating bacteria. They favor water with a low biochemical oxygen demand and they are mostly found in mills where the process water is

Table 5.1: Microorganisms commonly found in paper mills

Aerobic bacteria • Spore-forming • <i>Bacillus cereus</i> • <i>Bacillus megaterium</i> • <i>Bacillus mycoides</i> • <i>Bacillus coagulans</i> • <i>Bacillus amyloliquefaciens</i> • <i>Bacillus atrophaeus</i> • <i>Bacillus firmus</i> • <i>Bacillus fusiformis</i> • <i>Bacillus halodurans</i>, • <i>Bacillus jeotgali</i> • <i>Bacillus licheniformis</i> • <i>Bacillus megaterium</i> • <i>Bacillus mycoides</i> • <i>Bacillus pumilus</i> • <i>Bacillus simplex</i> • <i>Bacillus smithii</i> • <i>Bacillus sphaericus</i> • <i>Bacillus subtilis</i> • <i>Bacillus thuringiensis</i> • Non-sporeforming • <i>Achromobacter piechaudii</i> • <i>Achromobacter xylosoxidans</i> • <i>Acinetobacter radioresistens</i> • <i>Acinetobacter lwoffii</i> • <i>Acinetobacter baumannii</i> • <i>Aeromonas</i> • <i>Beggiatoa</i> • <i>Citrobacter</i> • <i>Corynebacterium</i> • <i>Enterobacter amnigenus</i> • <i>Enterobacter cloacae</i> • <i>Enterobacter hormaechei</i> • <i>Enterobacter kobei</i> • <i>Enterobacter radicincitans</i> • <i>Enterobacter sakazakii</i> • <i>Escherichia</i> • <i>Flavobacterium</i> • <i>Gallionella</i> • <i>Klebsiella</i> • <i>Leptothrix</i> • <i>Micrococcus</i> • <i>Pseudomonas</i>	• <i>Pseudomonas stutzeri</i> • <i>Pseudomonas aeruginosa</i> • <i>Pseudomonas fluorescens</i> • <i>Pseudomonas putida</i> • <i>Pseudomonas monteilii</i> • <i>Pseudomonas plecoglossicida</i> • <i>Pseudoxanthomonas</i> • <i>Sphaerotilus</i> • <i>Staphylococcus</i> • <i>Thiobacillus</i> Anaerobic bacteria • Spore-forming • <i>Clostridium intestinale</i> • <i>Clostridium magnum</i> • Nonsporulating • <i>Desulfovibrio</i> • Some <i>Actinomycetes</i> Fungi • Molds • <i>Alternaria</i> • <i>Aspergillus</i> • <i>Fusarium</i> • <i>Penicillium</i> • <i>Phialophora</i> • <i>Phycomycoses</i> • <i>Trichoderma</i> • <i>Mucor</i> • Yeast • <i>Candida</i> • <i>Geotrichum</i> • <i>Monilia</i> • <i>Rhodotorula</i> • <i>Saccharomyces</i> • <i>Torula</i> • <i>Oospora</i> Algae • Blue-green • <i>Asterionella</i> • <i>Navicula</i> • <i>Oscillatoria</i> • Green • <i>Chlorococcus</i>
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Based on Stanier et al. (1968); Sanborn (1965); Väisänen et al. (1989, 1991, 1994, 1998); Prince et al. (2009); Kashama et al. (2009); Suihko and Skyttä (2009); Suihko et al. (2005).

not recycled. One member of this group converts iron salts to iron oxide and deposits it in its external envelope. Another member of this group forms copious white filamentous plumules. Bacteria most often found in paper and board machine slime include species of *Flavobacterium*, *Clavibacter*, *Sphaerotilus*, and *Leptothrix*.

Slime/biofilm systems provide an anaerobic zone which enables the growth of sulfate-reducing bacteria: *Desulfotomaculum*, *Desulfovibrio* (Postgate, 1979). Sulfate reduction in biofilm systems is of worry in many water systems (Piluso, 1977; Robichaud, 1991). Sulfate-reducing bacteria produce hydrogen sulfide, which deteriorates concrete and causes corrosion of metals. In thick microbial films, heterotrophic organisms, denitrifiers, iron-reducing bacteria, sulfate-reducing bacteria, and methanogens may be where excess organic material, nitrate, iron, and sulfate are present. Sulfur bacteria make only a small contribution to the formation of deposits of sludge. One of their members—sulfate-reducing bacteria—is commonly found in the highly sulfurized water of paper machines. They reduce the sulfate ions in hydrogen sulfide and generate unpleasant odors and affect Fourdrinier wires and accessories made of copper alloy. They can also deactivate the mercurial slimicides by forming insoluble sulfurs. These bacteria grow poorly in the presence of gaseous oxygen but grow very well under the thick deposits of slime and those of fibers in the storage tanks of whitewater. Proteolytic bacteria break down the formulations of casein and gelatin used for sizing. *Pseudomonas* sp. are also in this category.

Most common fungal species found in slime are *Penicillium*, *Aspergillus*, and *Cephalosporium* (Brewer, 1960; Huster, 1992 a,b). The thermotolerant fungi play an important role in slime formation in paper mills in closed process water systems, where temperatures are usually maintained above 30 °C (Eveleigh and Brewer, 1963). Several fungal species—*Cladosporium*, *Geotrichum*, and *Mucor*—are also synergistic with bacterial species, forming hard slimes that are resistant to biocides and are very difficult to control (Sanborn, 1965). The extremely varied group of microorganisms produces an equally varied number of extracellular polysaccharides, reserve materials, and other excretory products that make up the slime. Unlike bacteria, fungi are not really at home in process water; as a result, they often form the fibrous mat on which the deposits of slime accumulate. To a large extent they are responsible for the worsening of balls of wet pulp in sheets in the course of stacking or of final paper products. The colored patches of the pulp are in general from colonies of *Basidiomycetes* and *Penicillium*. It is therefore important for the slime control program to include the use of fungicidal element. *Penicillium roqueforti* deactivates the mercury compounds.

Väisänen et al. (1998) performed a thorough examination of microbial communities of a printing paper machine. They isolated 390 strains of aerobic bacteria representing at least 34 species, thus demonstrating the large number of bacteria living in a single paper machine. The most frequent contaminants of the machine wet-end were species of genera *Bacillus*, *Burkholderia*, *Pantoea*, *Ralstonia*, and *Thermomonas*.

Raaska et al. (2002) observed that contamination of starch-based glues with spore-forming bacteria (*Bacillus* and *Paenibacillus*) and enterobacteria (*Citrobacter* and *Enterobacter*) was the most important factor threatening the process hygiene and product safety in one of the machines manufacturing refined paper products for food packaging. Mostly, the major contaminants of the dry paper products seem to be limited to three genera of spore-forming bacteria: *Bacillus*, *Brevibacillus*, and *Paenibacillus* (Hughes-van Kregten, 1988; Pirttijärvi et al., 1996, 1999; Raaska et al., 2002; Suominen et al., 1997; Väisänen et al., 1998).

Kolari (2003) conducted a detailed microbiological examination of colored biofilms in six paper and board machines, including two case studies of outbreaks of colored slimes in which the causative bacteria were found. They isolated a total of 95 pink-, red-, orange-, or yellow-pigmented strains. Most of the strains were found to be moderately thermophilic. About 99% of the strains grew at 52 °C and 72% grew at 56 °C, but only 30% grew at 28 °C. Microtiter plate assay was used to study the biofilm formation potential and the biocide susceptibility of the strains. Fifty strains were found to produce biofilm in the presence of 5 ppm of methylene bithiocyanate or 2,2-dibromo-3 nitrilopropionamide in paper machine water. Furthermore, 39 strains increased biofilm production by 5% to 753% in the presence of biocide. Five groups of primary biofilm-formers common in Nordic paper machines were recognized: *Deinococcus geothermalis*, *Meiothermus silvanus*, a novel genus of “*Rhodobacter*-like” alpha-*Proteobacteria*, *Burkholderia* spp., and *Thermomonas* spp. Many colored paper machine bacteria are species previously known from microbial mats in hot springs.

Chaudhary (1992) and Chaudhary et al. (1997) reported that *Bacillus alvei* and *A. aerogenes* were the most prevalent contaminants in an Indian paper mill. Oppong et al. (2000) isolated pink-pigmented bacteria *Deinococcus grandis*, *Flectobacillus* sp., *Methylobacterium zatmanii*, *Micrococcus* sp., and *Roseomonas* sp. from slime deposits of American paper machines. Harju-Jeanty and Väättänen (1984) showed that the filamentous bacterium *Sphaerotilus natans* can produce slimy aggregates in groundwater pulp (1%) at 28 °C, but it was not growing above 40 °C (Pellegrin et al., 1999), making it an unlikely biofilm-former in modern paper machines with operation temperatures near 50 °C.

5.2 Location of Slime on the Machine

Microorganisms can develop at any site of the water systems of a paper machine (Blanco et al., 1996). However, in actual practice, it has been found that they form at various points of relatively small isolated packets of proliferation. This depends on the combined effect of several factors. These are:

- Machine model
- Type of microorganism
- Chemical environment

- Water flow speed
- Pulp consistency
- Pulp composition
- Program for combating the slime

Even in the absence of heavy contamination, the construction type of the paper machine can exert a substantial influence on the formation of deposits of slime by supplying a multiplicity of points with zero flow. By this, the sectors of the machine in the pipe conduits where a certain degree of stagnation is produced are understood, for example: the inner side of the angles of pulp pipes, the T- or Y-shaped joints, the orifice plates, the rotary cleaners, cross-bars, basins and pulp lines, the tanks of rich white water, the wire rolls, the Fourdrinier, etc. All the efforts undertaken studying the machine for avoiding these dead points and making the interior of pipes smooth will contribute to reducing the problems caused by slime.

Bacillus cereus has been isolated from slimes in the wire section (Väisänen et al., 1989, 1998), whitewater, calendar water (Pirttijärvi et al., 1999), and steel coupons immersed in water circuits (Kolari et al., 2001). Suihko et al. (2004) found *B. cereus* from several paper mills. They isolated 27 strains of *B. cereus* from broke, slime, water, pulp, and chemical samples and from end-products. Thus, *B. cereus* is a common contaminant in paper machines and can be isolated from many different sites. From a bacterial point of view, paper machines resemble hot springs in many aspects: warm water is continuously running on solid surfaces. Bacteria isolated from both hot springs and paper machines are *Deinococcus geothermalis*, *Meiothermus silvanus*, *Pseudoxanthomonas taiwanensis*, *Rubellimicrobium thermophilum*, *Schlegelella aquatica*, *Tepidimonas* spp., and *Thermomonas hydrothermalis* (Väisänen et al., 1998; Kolari et al., 2001; Kolari, 2003; Peltola et al., 2008; Prince et al., 2009; Desjardins and Beaulieu, 2003; Suihko et al., 2004; Kashama et al., 2009; Denner Ewald et al., 2006; Tirola et al., 2009; Busse et al., 2002).

The nature of microorganisms developing in slime plays an important part in the location of slime on the machine (Purkiss, 1973; Blanco et al., 1996). Anaerobic bacteria are mostly found at points where the oxygen tension of the water is zero, under already existing deposits of slime or accumulations of fiber deposits. Anaerobic bacteria are also found in most other locations. Bacteria forming a thin gelatinous film propagate where the content of abrasive products of the water is at its minimum because, when the consistency of the pulp has risen (1–3%), the displacement of the cellulose fibers tends to remove the fine film as soon as it is produced. The pseudofilamentous bacteria need a certain turbulence and a surface suitable to affix on. These bacteria do not like substantial quantities of electrolytes and avoid points where the alum concentration is very high. Certain microorganisms can only produce coherent masses when they find a suitable mechanical base and produce colonies around the fibers, which when become too dense and tear off under their own weight. The fungi usually develop on the dried splashes situated above the water line of the wooden chests. If the

contamination is not substantial, the deposits of slime tend to form very far from the point of introduction but the opposite is not true when there is massive contamination.

Most mill infections are observed at a very localized, principal point, where the bacterial proliferation is able to develop and from where the greater part of the other infections expand. The whitewater circuit is the center of infection in most of the cases. The method mostly used for localizing the center of infection of a paper machine consists of conducting a quantitative microbiological examination, by using the plate count method. When this counting operation is carried out on a machine installation, it is mostly found that the highest concentration of microorganisms is in the buffer tank or in a whitewater storage tank, even if the deposits of slime are not apparent there. It appears that the microorganisms reproduce in the white water in which the conditions are favorable and take the form of slime where the chemical milieu is adverse. It is possible to predict the most favorable locations for microbial proliferation by biological means but the spores where slime forms seem to be a question relating to the hydraulics and type of machine.

The appearance of infections most commonly observed in a paper mill are the following (Purkiss, 1973):

- Formation of slime
- Blocking of the felts
- Degradation of the felt
- Fermentation of rosins
- Stains in the pulp
- Cellulolytic action
- Musty odors

The blackish, glutinous, and fibrous mass of slime shows its presence. The bacteria associate very rapidly with the cellulose fibers in suspension in the pulp as a result of their surface electrical charge. The effect of this association is to slow down ware removal in the wire. This variation in the rate of water removal is often the precursor of machine contamination. The most serious infections are shown by the appearance of slime stains in the final product.

The buildup of slime in the interslices of the fabric can very rapidly make the felt unusable until it is cleaned. Because the propagation of bacteria is progressive, the loss of yield will take place for a certain period before the complete working of the felt. When the microbial population of a machine contains proteolytic enzymes, the degradation of the fibers making up the woolen felts is obvious. This leads to a reduction in tensile strength properties and also the shear strength. Often the rupture of a felt is ascribed to normal mechanical wear, although a closer look shows that numerous points exhibit weak resistance, whereas purely mechanical wear is slight there. By the use of tanning, this problem can be reduced to a certain extent but not completely.

Rosin preparations constitute nutrients particularly suitable for microorganisms. The contamination of rosin can normally be found by the characteristic smell released. Protein-based adhesives emit a bad odor of ammonia or carbilamine, whereas starch normally smells like alcohol. Normally, these bad odors are not revealed until the paper is in the hands of the Converter. Rosins are contaminated in a fashion rather similar to that of the machine, but are problematic only if sufficient attention is not paid to the cleaning of the mixers or the rosin formulations are left for too long.

The pulp tends to promote the development of fungi rather than the bacteria because the pulp rarely contains a great deal of free water. Proliferations of fungi are often pigmented and appear in the form of stains in the pulp. It may also be the case that these stains appear only after a certain period of storage. That mercury compounds have been added does not provide any guarantee in regard to nonappearance of these stains, as there are certain fungi such as *P. roqueforti* that are capable of absorbing mercury. Significant research has been conducted on this microorganism. It can reduce the concentration of mercury in the sheet of pulp to such an extent that the development of other types of microorganisms becomes possible.

The effects of the action of fungi and cellulolytic bacteria are not generally felt in the mill itself. The effect of their attack is not apparent in the paper until it reaches the final consumer because these microorganisms degrade the cellulose and reduce the tensile strength of the fibers. In the case of products, for example kraft racks of several thicknesses, this problem can mainly be resolved by paying attention to slime control or, in some cases, by subjecting them to a treatment to make the product nonbiodegradable. Complaints concerning the development of mold on the finished products are very common. Many things can be carried out on the machine to stop the proliferation of mold on papers for special purposes.

Generally, disagreeable strange odors are associated with the various phases of the production of paper and board. The most common of these odors are the following: first is the musty, earthy smell, which is normally emitted from stacks and covered tubs, and finally the offensive smell of hydrogen sulfide, which is often noticed when the channels under the machine are cleared. These are result from the manifestations of microbiological activity. The musty, earthy smell is normally from proliferation of fungi under the lids of the stacks. Hydrogen sulfide results from the action of sulfate-reducing bacteria in conditions where there is only little or no free oxygen.

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Chemistry of Deposits on Paper Machines

Modern papermaking is, from a mechanical point of view, a very well-refined process using what might well be the most advanced process control used in any industry. However, the modern papermaking process is very sensitive to disturbances, and the formation of deposits is one of the most important factors limiting the productivity on a paper machine today (Hassler, 1995). Further, paper defects (such as breaks, holes, and spots very often caused by deposits) represent one of the most common complaints of a user/buyer of paper. Recycled waste paper often contains large quantities of nonfibrous material causing a deposit problem. Further, it is only natural that microorganisms that potentially can cause problems in the papermaking process are present in a papermaking system because these represent a natural part of life with presence just about everywhere on Earth.

Papermaking deposits can be classified as:

- Organic deposits
- Inorganic deposits
- Microbiological deposits

Each one presents its particularities according to their composition. In practice, most paper machine deposits will contain a combination of some or all of these major component types. Although inorganic deposits tend to be more of a problem in the stock and approach flow piping systems of the paper mill, organic deposits tend to be more problematic in the headbox, wet-end, and press section of the paper machine. These contaminants form tacky deposits that can plug forming fabrics and wet press felts. Examples of common organic deposits found in paper mill systems include: wood, pitch, sizing agents, starch, defoamers, wax, ink, latex, and wet-strength agents.

Microorganisms in paper mill systems take two forms: planktonic (free swimming) and sessile (attached to a surface). Planktonic organisms do not readily cause problems in the system; however, in the sessile form these same organisms can become problematic (Costerton et al., 1999; Donlan and Costerton, 2002; Dunne, 2002). Inorganic and organic deposits in the system offer a readily available food source for sessile organisms. They are able to attach themselves to deposit surfaces, and a number of these organisms classified as slime formers are able to secrete a protective polysaccharide coating around themselves. This coating, known as biofilm, is very gelatinous in nature and easily traps other contaminants in the system. It is also very resistant to standard chemical treatments used to control biological

organisms. The slime formers, in combination with inorganic and organic process additives and contaminants, can form deposits that coat process equipment. These deposits create sheet quality and runnability problems. In addition to slime formers, anaerobic bacteria can form underneath deposits. The most common type of anaerobic bacteria found in paper mills is sulfate-reducing bacteria. This type of organism is capable of converting sulfate in the system to highly corrosive hydrogen sulfide.

The conditioning film is considered to be important to the formation mechanism of a deposit (Schenker et al., 1998; Kolari et al., 2001). It is important to understand the origin of that layer and the building of the bulk deposit to design an efficient treatment program (Kanto Öqvist et al., 2001). The particle size has an important effect on the deposit formation. Persson (2004) has demonstrated the classification of the constituents present in the paper machine circulation water by their particle sizes. Klein and Grossmann (1995) report that a major part of the substances found in deposits are colloidal substances.

6.1 Organic Deposits

Several researchers have reported that the alkaline sizing agents—alkyl ketene dimmer (AKD) also called AKD wax and alkenyl succinic anhydride (ASA) are commonly associated with the fouling of paper machines, both in the wet-end and the dryer section (Knubb and Zetter, 2002; Koskela et al., 2003; Nguyen, 1998; Petander et al., 1998). The strong likeness of sizing agents toward precipitated calcium carbonate is widely known (Petander et al., 1998) and the tacky organic materials are known to be included in organic deposition. These typically originate from various sources (Alén, 2007; Negro et al., 1999):

- Virgin pulp
- Recycled pulp
- Broke
- Additives
- Adhesives
- Waxes

Most of these compounds originate from the wood and are called extractives. These are either soluble in different nonpolar solvents—diethyl ether, dichloromethane or methyl tert-butyl ether, acetone, ethanol, hexane, toluene, and tetrahydrofuran—or are soluble in water (Alén, 2007). Extractives and resins are both used as collective names for all lipophilic components most of the time. These can be extracted from wood by nonpolar organic solvents. Different types of extractives are essential to maintain the different biological functions of the tree. Lower terpenoids, resin acids, and phenolic substances protect the wood against microbiological damage or insect attacks. Fats constitute the energy source of the wood cells (Sjöström, 1992). The deposits on paper machines generally contain a mixture of most of these substances (Hubbe et al., 2006). Tacky materials are hydrophobic and insoluble in water. Their deposition behavior

is controlled by temperature, hydrodynamic and mechanical forces, and changes in chemical environment (pH, ion concentration) (Allen, 1980; Back and Allen, 2000; Carré et al., 1998; Dreisbach and Michalopoulos, 1989; Klein and Grossmann, 1995; Monte et al., 2004). There are significant differences in the amount and type of extractives originating from chemical pulping, mechanical pulping, and recycled fiber (Alén, 2007). The classical coagulation theory of organic colloids - Derjaguin-Landau-Verwey-Overbeek, also called DLVO considers the following interactions between similar dispersed particles (Kallio et al., 2004; Alén, 2007):

- Attractive van der Waals' interaction and
- Repulsive electrostatic interactions

The problems caused by these extractive materials are worse when the water systems are closed because of the buildup of contaminants (Monte et al., 2004).

6.2 Inorganic Deposits

Dissolved compounds such as polyelectrolytes, surfactants, or ions may form insoluble salts or complexes as a result of changes in concentrations, pH, temperature, and process conditions. They can be present as stable colloids or as macroscopic phases (Merta, 2001) that form particles that are larger than colloidal size, depending on pH, temperature, gas content, concentration of substances, and ionic strength. Barium sulfate deposits have been found on screens, cleaners, fan pumps, organ tubes, headboxes, rectifier rolls, headbox lips and slices, and on Fourdrinier foils. The problem is more severe when hardwood is present. Alum is used in acid papermaking for several applications such as pH control, pitch control, retention, water clarification, and rosin sizing. Some alum is frequently used in alkaline papermaking to improve sizing by increasing fines retention and also to improve retention and drainage. When the pH of a system exceeds 5.0, the alum hydrolyzes to form insoluble aluminum hydroxide that can cause serious deposit problems. The deposits occur in various places such as primary screens, fan pump blades, machine chests, headboxes, wire return rolls, foil blades, vacuum boxes, and couch rolls. Other aluminum-based deposits that develop include aluminum phosphate and aluminum-size deposits of both hydrolyzed ASA and AKD. Soluble surfactants form insoluble salts (soaps) with di- or multivalent metal ions (Holmberg, 1999; Allen, 1988). The formation of inorganic salts from ions is a relatively common fouling mechanism, since the processing waters are very often saturated with ions. Hydrophilic interfaces are commonly assumed to be the best sites for nucleation of inorganic salts (Brecevic and Kralj, 2000).

6.3 Biological Deposits

The vast majority of microorganisms live and grow in aggregated forms such as biofilms and flocs. This mode of existence has resulted in the generally accepted term: biofilm. Bacteria stick firmly to nonliving surfaces, initially in reversible association and ultimately in an irreversible

adhesion (O'Toole et al., 2000; Marshall et al., 1971; Costerton et al., 1987; Watnick and Kolter, 2000) and initiate the development of adherent bacterial biofilms. The quality of the surface is very important for the attachment and depends on hydrophobicity and hydrophilicity of the surfaces. Several researchers have explained that the mechanism of adhesion are glycocalyx polymers (Costerton et al., 1987; Bryers, 2000; Kolari et al., 2002; Saarimaa et al., 2006; Parkar et al., 2001; Ghannoum and O'Toole, 2004; Peltola et al., 2008). These show that glycoproteins may be involved in the initial attachment, followed by aggregation and microcolony formation and possibly ending in biofilm formation by secretion of extracellular polymeric substances (Ghannoum and O'Toole, 2004). Biofilm acts as a protective layer against potentially harmful agents as well as a carbon and energy source at times of nutrient deprivation, resistance to ultraviolet light and increased rates of genetic exchange (O'Toole et al., 2000). The organic matrix of a biofilm acts as a habitat for the microorganisms and serves several functions which include holding the biofilm together and providing a favorable environment for growth and long-term survival of the resident cells. This organic matrix also provides a local environment in which the cells are protected from extreme changes in physiochemical parameters of the water. Moreover, the matrix is important in sequestering nutrients from the water column and protecting the cells from drying if the water level decreases. Table 6.1 demonstrates the thermal conductivity of a variety of deposit-forming compounds in contrast to biofilm. A lower number indicates a greater resistance to heat transfer.

Studies show that there are significant differences in biofilm-forming capacity of bacteria (Lindberg et al., 2001a,b), and although some general concepts can be applied, many species-specific behaviors exist that reflect the special needs of each microorganism (O'Toole et al., 2000). Mack et al. (2006) reported that there is a need for the paper industry to understand the mechanism of biofilm formation and also to understand to prevent biofilm problems associated with medical devices and how they cause infections. Spores may also attach to stainless steel in greater amounts than vegetative cells (Parkar et al., 2001). The majority of studies on the attachment and biofilm formation mechanism have been conducted using *Pseudomonas aeruginosa* and *Staphylococcus epidermis*. Raulio et al. (2008) have reported that the mechanisms are species-specific and depend on the type of surface. They described an

Table 6.1: Thermal conductivity comparison of biofilm and deposit-forming compounds

Substance	Thermal Conductivity (W/m K)
Biofilm	0.6
Calcium sulfate	2.3
Calcium phosphate	2.6
Calcium carbonate	2.6
Iron oxide	2.9
Analcite	1.3

Based on <http://www.wet-usa.com/data/bullets/46.pdf>.

adhesion mechanism for *Pseudoxanthomonas taiwanensis* involving cell ghosts on which the biofilm grew. Studies conducted with *Deinococcus geothermalis* showed that the adhesion mechanism is mediated by adhesion threads (Kolari et al., 2002; Peltola et al., 2008; Raulio et al., 2008). Kolar (2003) has studied attachment mechanisms and properties of bacterial biofilms on nonliving surfaces. Korber et al. (1995) reported that microbes develop different types of attachment behaviors once in initial contact with a surface. Motile attachment behavior of *Pseudomonas fluorescens* allows the flagellated cells to move along surfaces in a semiattached condition within the hydrodynamic boundary layer, independent of the flow direction. Vigeant and Ford (1997) reported reversible adhesion of *E. coli* cells with residence times of more than 2 min on a surface as near-surface swimming. When microbes can no longer move perpendicularly away from the surface, the term irreversible attachment is used (Busscher et al., 1998). Most microorganisms irreversibly attach only after a period of unstable, reversible adhesion, during which the cells can show motion (Korber et al., 1995). *E. coli* and *Vibrio cholerae* first use the flagella to spread across the surface, and then anchor onto the surface with pili and the outer membrane proteins. *P. aeruginosa* needs type IV pili for twitching motion on a surface and for the subsequent buildup of stagnant microcolonies (O'Toole et al., 2000).

Kolari (2003) describes that microbes can attach irreversibly while retaining active motility by mechanisms known as gliding, swarming, twitching, swimming, darting, and sliding. Harkes et al. (1992) reported that uropathogenic *E. coli* cells attached irreversibly and still actively migrated along solid surfaces. Biofilm structures in mature biofilms are firmly cemented to their place on a surface until they age and detach. This observation was challenged by Stoodley et al. (1999). Time-lapse microscopy showed downstream migration of ripple-like biofilm structures of mixed-species growing in turbulent flow. A study by Tolker-Nielsen et al. (2000) showed that cells of *Pseudomonas* sp. move actively by their flagella inside and between the microcolonies of a developing biofilm. Several studies exist in which bacterial attachment has been studied with different bacterial species, on different surfaces, and in different ionic strength, flow, or nutrient conditions. The adhesion of *Bacillus* spp. has been studied by many researchers. Husmark (1993) reported that *Bacillus* spores adhered as monolayers on many kinds of surfaces. The hydrophobic spores of *Bacillus cereus* were found to be most adhesive. Surfaces or surface coatings that hinder bacterial attachment have been described but none has been developed that totally prevent it (Dunne, 2002). Bacteria are living organisms that become accustomed to the requirements set by their changing environment and alter their surface composition. Bacterial cell surfaces are mostly hydrophobic, but the cells can possess hydrophilic appendages. The net charge of most bacteria is negative, but Dunne (2002) has reported that *Stenotrophomonas maltophilia* has positive surface charge at physiological pH. The cells can also have polarity in their charge. In a field of direct current, the rod-shaped cells of *Actinomyces naeslundii* adhered flat on the indium tin oxide surface. In an alternating current field, the cells adhered parallel to the field (Poortinga et al., 2000). There are several reports that showed that adhesiveness of bacteria also varies with different growth phases (Flint et al., 2001; Husmark, 1993; Peng et al., 2001). Finally, the surface properties of any

novel colonization-resistant surface will be gradually changed by adsorption of salts, proteins, glycoproteins, and other molecules from the environment. Other organisms such as fungi, diatoms and other algae, amebae, and ciliates can also be found in biofilms. Their quantity depends on the specific environment. Within biofilms in drinking-water distribution systems, *Legionella* spp. may be enclosed in cysts of amebae and be protected from chlorination (Szewzyk et al., 2000). Fungal biofilms have not received much attention except for the clinically important species. Dimorphic yeast *Candida albicans* has been commonly isolated from medical devices (Kumamoto, 2002). It forms biofilms. Similar to the biofilm of bacteria, the most important property of the fungal cells is their increased resistance to antibiotics. Kumamoto (2002) reported that the resistance may arise from contact-dependent expression of drug efflux genes not expressed in planktonic cells. Biofouling of industrial processes is often caused by mixed-species biofilms. Jones (1995) reported the presence of fungi in these biofilms. Strains of *Fusarium solani*, *Fusarium oxysporum*, and *Rhodotorula glutinis* isolated from photo-processing industry were found to form biofilm as pure cultures and in mixed cultures with bacteria (Evers et al., 1998, 2002). In comparison to single-species biofilms, mixed fungal–bacterial biofilms showed greater protection against isothiazolone biocide. Mechanisms for the undesired persistence of *Bacillus* sp. in paper machine slimes were investigated by Kolari et al. (2001). Biofilm formation was measured for industrial *Bacillus* isolates under paper machine wet-end–simulating conditions. None of the 40 tested strains of seven *Bacillus* species formed biofilm on polished stainless steel or on polystyrene surfaces as a monoculture. Under the same conditions, *Deinococcus geothermalis* E50051 covered all test surfaces as a patchy thick biofilm. The paper machine bacilli, however, formed mixed biofilms with *D. geothermalis* E50051 as revealed by confocal microscopy. Biofilm interactions between the bacilli and the deinococci varied from synergism to antagonism. Synergism in biofilm formation of *D. geothermalis* E50051 was strongest with *Bacillus coagulans* D50192 and with the type strains of *B. coagulans*, *Bacillus amyloliquefaciens*, or *Bacillus pumilus*. Two *B. licheniformis*, one *B. amyloliquefaciens*, one *B. pumilus*, and four *B. cereus* strains antagonized biofilm production by *D. geothermalis*. *Bacillus licheniformis* D50141 and the type strain of *B. licheniformis* were the strongest antagonists. These bacteria inhibited deinococcal growth by emitting heat-stable, methanol-soluble metabolite(s). It was concluded that the persistence of *Bacillus* sp. in paper machine slimes relates to their ability to conquer biofilms formed by primary colonizers, such as *D. geothermalis*.

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*Methods for Determining the Microbiological Contamination Level**

For achieving optimum microbiological control, the complete microbiological investigation of the mill is required. This study aims to determine the infection level of the plant and its origin. It consists of a complete analysis of the system. This study is applied to the paper machine, in the coating section, in starch preservation, and with odor problems in the final product. The microbiological investigation in a paper mill starts with the study of the whole system; collecting information about the process and the paper machine. The critical areas of the process are the wet-end, coating section, and the size emulsion (Blanco et al., 1997). The deposits are analyzed microscopically to identify the type of infection in the different parts of the machine. The infection level and the source of contamination is determined by analyzing the water from the pulper, headbox, whitewater, clarifier input and output, brokes, kaolin, calcium carbonate, and starch, etc. In the wet-end, it is important to check the machine frame under the wire, the surface of the foils, the suction boxes, the whitewater tanks, and the clarifiers for any possible presence of deposits. If deposits are present, the smell, texture, and color should be examined. In the coating section, the presence of a bad smell indicates microbiological problems. Several different techniques have been reported for the study of the microbiological contamination by various researchers (Farkas et al., 1987; Farkas, 1990; Prasad, 1989a,b; Salzburger et al., 1990; Heck and Hollis, 1984; Guerin, 1983; Young-Bandala and Boho, 1987; Barclay, 1994; Wolfaardt et al., 1993). Table 7.1 presents various physical, chemical, biological, and optical measurement methods used in the paper industry for biofouling.

7.1 Off-Line Methods

7.1.1 Slime Collection Boards

Slime collection boards are placed in the water of a paper machine in a way that will cause slime formation. These boards are inspected at frequent intervals, which makes it possible to get the formation of slimes on the machine in the early stages before their presence become problematic. Several authors have studied the use of these boards. The most effective and less costly ones are made of plastic (Plexiglas). Small rectangular pieces of Plexiglas are used and two holes are pierced so the pieces can be suspended. These pieces are slightly curved and placed in a flow of water in such a way that an area of some turbulence is created on the concave side. It is

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Table 7.1: Methods used in the paper industry for biofouling

Physical methods
Oxygen depletion
ATP measurement
Slime measuring board
Microcalorimeter
pH and redox measurement
Volatile fatty acids
Sulfide measurement
Chemical methods
Ninhydrin test
Resazurin test
Dehydrogenase activity
Fluorescent redox stain
Genetic methods
Fluorescence in situ hybridization test
Polymerase chain reaction
Immunofluorescence marking
Biological methods
Cell count determination
Luminescent bacteria test
Minimal inhibitory concentration
Hemmhof test
Bacteria identification

Based on Hilbert et al. (2001).

easy to notice the formation of slime on a collecting board. The extremely fine coating gives a greasy feeling. These slime boards must be placed in any locations as necessary and should be inspected regularly. According to Hilbert et al. (2001), the only specified method that attempts to quantitatively determine biofilm growth is the slime measuring board. The performance of the slime measuring board is more or less dependent on several factors, like the design of the measuring cell, the selected surface of the measuring board, and the method of evaluation.

7.1.2 Identification of the Contaminated Points

It is important to develop some method that can distinguish the marks of biological origin from the others because the stains and the black area of the paper are basically not caused by slime. Most paper mills do not have microbiologists; conventional microbiological methods for stain detection have not received not much interest. Therefore, chemical methods are used. The ninhydrin test and tetrazolium chloride test have been developed in the past few years.

7.1.3 Standard Plate Count Method

This is a generally accepted method for quantifying biological activity in a system. Serial dilution of the sample is undertaken, and the sample placed in a sterile nutrient medium in a

petri plate. Normal paper mill populations range from less than 10,000 organisms/mL of sample to more than 100 million organism/mL. The plates are incubated and the individual micro-organisms are allowed to reproduce in the solidified nutrient medium. The incubation time for bacteria is 48 or 72 h, whereas for fungi it is 5–7 days (Cloete et al., 1992). This method has many technical limitations that are overcome using slightly modified methods or nutrients.

7.1.4 Rapid Counts

Some rapid tests have been developed to avoid the limitation of the traditional plate count method. When great accuracy is not required, dip sticks are used. These are marketed by a number of companies and contain a solid nutrient and a color-changing indicator, which allows colonies to be counted. The device is dipped into a water sample for a short time. The number of colonies on the surface of the device is counted after incubation for 24–48 h. A comparison can also be made with a chart to measure a color change, which is then correlated to standard plate counts. This method gives a rough indication of the biological activity present in the sample. The rapidity of the test is a great improvement over the standard plate count method. Petrifilms are used when greater accuracy is required or when the contamination level is lower than 10^3 bacteria/mL. The spiral plate count method is also used for selecting biocides.

Mentu et al. (2009) have reported a portable microbiological enrichment unit for microbiological control in a paper machine. The device is a case that eliminates a need for a microbiological laboratory. Even though this is a good effort toward rapid analysis, it requires at least 24 h and is thus still too slow. However, the whole procedure appears easier and gives additional information compared with the conventional plating method.

7.1.5 Adenosine Triphosphate Assay

The quantification of adenosine triphosphate (ATP) has been used as a bacterial cell viability assay method (Kramer et al., 2008; Najafpour, 2007). ATP is the molecule associated with all biological activity; therefore, it can be considered a biological indicator. The amount of ATP in cells is directly connected to the activity status of the cells. Metabolically active cells have a higher ATP content than starving cells in an environment rich with nutrients. Bacterial spores have a very low ATP content, so an ATP assay will overlook spores in the process samples. ATP is stable in the pH range of 7–9, but will quickly decompose in acidic conditions; as a result, ATP analysis cannot be used in monitoring acidic processes. Mentu et al. (1997) reported that biomass measurement using an ATP assay gave results immediately but was found to be suitable only for high levels of microbial contamination because of the low sensitivity of the assay. The sensitivity of ATP assays has improved in the recent years. Several portable devices have been developed that make ATP measurement easy to perform.

The results of the ATP assay are received in less than 1 min, whereas in the traditional method, 3 days are required. These devices are mainly used for the hygiene monitoring and have not yet been widely used in papermaking.

Because different species of bacteria and fungi function with different levels of ATP, a comparison with plate counts on a stable population should be performed before the luminescence measure has meaning. There are different methods available for ATP measurements; among them, an enzymatic method based on bioluminescence is the most sensitive. The easiest and the most rapid to use is a portable luminometer with different sampling dippers. This system was designed for hygiene monitoring, mostly in the food industry. The correlation between ATP and the total bacterial count in paper broke have been studied in pilot trials (Kiuru et al., 2011). The ATP content correlated with the total amount of aerobic heterotrophic bacteria.

7.1.6 Bio-Lert Method

The Bio-Lert method has provided accurate, valuable information about biological activity in pulp and paper mill systems on a timely basis, leading to raw material cost savings, continued up-time, and greater peace of mind. This method uses an indicator system with a sterile stabilized nutrient broth in a convenient vial. The liquor portion of a paper mill sample—stock, filler slurry, starch slurry, coating—is poured into the vial, filling it to the 10-mL mark. The vial is capped and the contents are shaken to thoroughly mix the nutrient and the indicator with the contents. The vial is then incubated at 37–42 °C. The time required for the color change from blue to pink is correlated to standard plate counts. Laboratory testing has shown that the time for a sample to cause color change is dependent upon the biological population in the original sample. Population in the range of 100 organisms/mL to 1 billion organisms/mL have been studied. Using the Bio-Lert vials, excessive biological populations can be detected very quickly, in as little as 1 h. This provides an early warning of biofouling and can be used to take corrective action before damaging slime buildup occurs in the industrial process.

This method has been found to be effective and stable despite wide variation in incubation temperatures or sample pH environments. The optimum incubation temperature is 37–42 °C. Several sample types are found to respond, including wood pulp fiber suspensions, filler slurries, and starch slurries.

A comparison with standard plate counts should be made for each sample point to improve accuracy because different micro-organisms may react differently in the test. The test is intended to provide an alert to high levels of biological activity. When systems containing low levels of biological activity are studied, it takes several hours for the color change to occur. If a sample produces unusually rapid color change, a biological control upset is indicated. Then, the cause of the upset can be found and corrective measures are taken before there has been an economic impact upon the mill.

7.1.7 Slime Monitor

A slime monitor has been developed by Sugi (1999). This monitor allows on-line, real-time measurement of slime deposition and makes sure that the minimum possible amount of biocide is used to the greatest possible effect.

7.1.8 Other Methods

Kolari et al. (2003) describe a promising method of how a microtiter plate assay can be used in studying biofilm growth tendencies and how biocides influence biofilm at the laboratory scale for the paper industry.

Álvarez-Barrientos et al. (2000) reported using the confocal laser scanning microscope to analyze biofilms in the paper machine loops, and how complex the matrix can be, but did not explain how the sampling should be done. Using flow cytometry and fluorescence in situ hybridization is also described, but is still under development for the paper industry (Nohynek et al., 2003; Torres, 2008). Several different positive and negative outcomes are described with respect to different microscopic methods used for biofilm analysis (Wolf et al., 2002).

Kemira has developed a new monitoring technique targeted at measuring the primary-biofilm formers called the pigmented biofilm-forming bacteria assay. It uses a proprietary cultivation media, either in the form of agar plates or in a dry plate form for field use. The medium does not contain any indicator chemicals often used to facilitate counting (i.e., the reductive dye in 3M Petrifilm™). Most of the identified primary biofilm formers, such as *Meiothermus* species, are naturally pigmented micro-organisms. This is exploited in their detection by counting colonies matching a color criterion under specified growth conditions. Mill studies have confirmed the method is useful for detecting the presence of pink/red, primary biofilm formers and quantifying changes in their amounts, information that was unavailable using traditional methods (Keegan et al., 2010).

7.2 On-Line Methods

To avoid economic losses associated with quality and runnability issues, methods supplying continuous on-line information of the actual tendency of the deposit formation are essential to be able to optimize and extend the amount of countermeasures (Flemming, 2002, 1998; Klahre et al., 1998). Only if the formation of such deposits is noticed in the initial stage, can efficient and cost-effective counteractive measures be triggered. Several types of deposit monitors are available based on light measurements sent through a glass surface (Swapan and Farr, 1997). Exceptions from the glass surfaces are the bypass units with steel plates reported by Korhonen and Väättänen (2002); the device is based on direct measurement during the process. It is believed to be the only one that does not use a bypass and includes the conventional slime board originally made of wood, which is popular in Germany (Klahre et al., 1998; Hilbert et al., 2001).

Indirect methods have been used for monitoring microbiological activity. These methods are based on analysis of volatile compounds such as fatty acids, hydrogen sulfide, and halogens from the biocides, etc. The compounds are detected using a special type of sensors (Savolainen and Pitkanen, 2000; Keppler et al., 2007; Lindberg, 2005). These methods offer continuous on-line monitoring and even a possibility to build control loops with the measurement. There are several installations at paper machines that have not become successful because of laborious maintenance and complex data interpretation.

Buckman Laboratories has developed a dual-channel biofouling monitor that can be used to measure buildup of slime in whitewater and cooling water circuits. This instrument provides a quantitative measure of slime accumulation and can be used to optimize microbial control programs in paper mills. The monitor shows a numeric and graphical index of biofouling and incorporates nutrient augmentation and other technology to moderate sensitivity to slime buildup. This allows the mill to take remedial action before the effects of potential problems are translated into spoiled raw material or lost production. The features also allows the sensitivity of the device to be adjusted to correlate the monitor output with paper machine performance. This instrument was used in an alkaline fine paper mill to monitor fouling following a wet-end boilout. It was noted that biofouling in the nutrient augmented channel was increased by several days in comparison with whitewater only (control). The data were modeled with an exponential curve. The specific fouling rate of 1.27 d^{-1} was obtained for the accelerated channel and 1.03 d^{-1} for the control channel. ATP measurements and microscopic analysis showed the buildup of slime.

Kolb has developed BioDeposit Control, which measures thickness of the biofilm. The device can be operated on-line, which enables early detection and quick response to problems (Bunk et al., 2006). The measurement principle is based on deposition's insulating feature when the deposit is in contact with water medium.

Another tool that has been developed to monitor the presence of biocides is the Ondeo Nalco TRA-CIDE (Simons et al., 2003), which measures the light reduction over a fixed period that is released by a suspension of proprietary bacteria that are in contact with process water. The decrease in light emission is proportional to the amount of bacteria that is killed. The readings can be converted into relative toxicity units (RTU). The higher the RTU value, the higher the biocidal activity in the sample. The TRA-CIDE toxicity measurement can be used to carry out a cycle study to check the potential efficacy of a biocide that is slug dosed in a system in time and place. Thus, it can be used to optimize a feeding strategy and/or to demonstrate the potential efficiency of a microbial control program.

Nalco has developed OxiPRO system, which obtains real-time data on deposit formation and microbial activity from the paper machine. The OxiPRO Biomonitor and Activity Monitor are sophisticated instruments designed to obtain the maximum benefit from Nalco biocontrol programs. The OxiPRO Biomonitor quantifies the rate and extent of surface fouling. The Activity Monitor incorporates bulk water and surfaces in a measuring device that records microbial activity. Because data are collected continuously using large volumes of process water, the

dynamic process conditions are better represented. Readily accessible data allow for rapid, proactive control in response to process changes. Biofilm growth, optical fouling, redox potential, and pH are measured. The analysis is carried out in separate software (TrendGen) using multi-variate tools (Meier, 2009). This is compared with conventional techniques that provide only an infrequent or partial assessment of the level of control.

Ashland Hercules' Decutec method is based on the microscopic analysis of coupons. An on-line unit is also available that monitors deposits on a stainless steel surface. A sensor is placed in a bypass chamber or built into any chest or channel in the paper machine. By analyzing light scattering in the deposit layer, data on its thickness, growth speed, and density are obtained (Jerusik and Davis, 2010).

One tool for on-line and real-time monitoring of the deposition or fouling rate is Oondeo Nalco's disk Optical Fouling Monitor (OFM) developed by Wetegrove and Banks (1992). The instrument displays a fouling index in time, which is a relative measure of the deposition in time. Another tube-type OFM has been invented by Banks and Wetegrove (1993) and further developed by others (Klahre and Flemming, 2000). Both instruments are very useful in providing on-line data of paper machine fouling that correlates well with holes and breaks. These instruments provide a tool to optimize, improve, and/or adjust an existing program and also have been used to decide when a system clean-up (boil out) is required (Simons et al., 2003). The OFMs can also be used to monitor an on-line dispersant's efficiency on the machine. However, for selection of the optimal dispersant, it is advantageous to be able to measure deposit control efficiency in a side stream rather than in the process. For this purpose, a simple mobile bypass instrument has been developed that consists of two identical pipes through which a constant flow of process water is passed. By dosing different deposit control chemicals to one of the pipes, the deposition can be easily compared. This allows evaluation before an actual machine trial and can be used to compare several dispersants. Combinations of dispersants, oxidants, and/or biocides can also be investigated before an actual trial. Interestingly, such studies revealed that laboratory tests do not always give the right answer on what would be the best dispersant in a real process.

Robertson and Walker (2000) have reported a specialized photometer unit for on-line biofilm monitoring. This allows rapid assessment of microbial metabolism by monitoring ATP. The unit's diagnostics are also used to monitor relative levels of biocides in mill fluids. Collection of data is possible within minutes, rather than days, with these tools. This allows rapid changes to the control program.

All on-line biofilm monitoring techniques are based on some kind of signal obtained from the biofilm under investigation (Angell et al., 1993; Fonseca et al., 2002; Freitas dos Santos and Livingston, 1995; Helle et al., 2000; Heydorn et al., 2000; Holtmann and Sell, 2002; Jass et al., 2001; Kappelhof et al., 2002; Klahre and Flemming, 2000; Knudsen, 1981; Le Bras et al., 1998; Leitz et al., 2002; Lewandowski and Beyenal, 2003; Li and Bishop, 2003; Markx and Kell, 1990; Mollica and Cristiani, 2003; Nivens et al., 1993, 1995; Pereira et al., 2003;

Philip-Chandy et al., 2000; Pinheiro et al., 1988; Pryfogle et al., 2002; Rudh et al., 2002; Saxena et al., 2002a,b; Schmid et al., 2001, 2002, 2003; Tamachkiarow and Flemming, 2003; Tinhaam and Bott, 2003; Vanhooren et al., 2002; Wetegrove and Banks, 2000; Wolf et al., 2002; 2003; Xavier et al., 2000, 2003). Usually these signals constitute some kind of energy transfer:

- Radiation (including light)
- Acoustic waves
- Electrical fields
- Electric current
- Heat transfer

Some of these signals are actively released by the biofilm, such as bioluminescence, but the huge majority is a passive response to input signals that were generated by the monitoring equipment. In the actual process of biofilm monitoring, these input signals are transmitted to the investigated surface, modified by the biofilm (if present) and its environment, detected by specific sensors, and usually processed in more or less sophisticated ways. It is in the modification of the signal that the biofilm leaves its characteristic footprint. Signal features that may be modified include the following (Janknecht and Melo, 2003; Lie et al., 2002; Pauly and Snaidr, 2001; Kallio, 2004; Kallio and Kekkonen, 2005):

- Intensity of light
- Intensity of sound
- Color/wavelengths
- Mechanical resonance frequencies
- Electrical capacitance
- Electrical conductivity
- Light refraction indices
- Friction
- Thermal resistance
- Optical input signals that are being modified into acoustic output signals

Almost all the mentioned techniques allow continuous monitoring of biofilm formation, and automatic data collection is easy to implement. This allows the setup of real-time, on-line methods for preventing biofilms with measures connected to dosage of different deposit control products and biocides. Table 7.2 summarizes the individual methods' characteristics.

Klahre and Flemming (2000) have described differential turbidity method. They analyzed slime or biofilm formation by differential turbidity and pressure drop measurements in the mill, giving on-line information of total slime formation but not allowing for analysis of the biofilm architecture. This method is very simple and efficient and uses the absorption and scattering of light by a biofilm. The setup basically consists of two turbidity meters that monitor the turbidity in a whitewater stream of a paper mill process. While the glass windows of one turbidity meter is regularly cleaned by a water jet, the other one accumulates deposits

Table 7.2: On-line biofilm monitoring methods

Method	Detection Limit
Differential turbidity method	0.1 mm
Fiber optical switch	105 cells/cm ²
Heat transfer	–
Pressure drop	–
Metabolic products	–
Computerized image analysis	Individual cells
Bioluminescence	–
Fluorometry	–
Spectroscopy	0.05 mm
Attenuated total internal reflection	–
Nuclear magnetic resonance spectroscopy	–
Photoacoustic spectroscopy	10 m resolution
Electrochemical techniques	–
Impedance measurements	0.2 mm
Capacitance measurements	108 cells/cm ²
Vibration sensor in solid surface	3 × 105 cells/cm ²
Vibration sensor in liquid phase	–

Based on [Janknecht and Melo \(2003\)](#).

which increases the measured turbidity value. The difference of the two instruments' readings thus corresponds to the accumulation of deposits between them. However, this method is not able to detect biofilm thickness that is less than 0.1 mm, which might be limiting in some applications. Practical problems concerning the water jet cleaning process can however occur.

[Wetegrove and Banks \(2000\)](#) reported a similar method using mechanical wipers instead of water jets for cleaning the reference detector. These researchers also presented a similar method in which light is conducted through fiber optics and divided into two separate beams. The first beam passes through a liquid streaming in a transparent tube, whereas the second one only passes through the free flowing system. Because the biofilm can only form inside the tube, the difference in light reduction between the two beams is considered to correspond to its growth.

A technique based on a fiber optic probe has been reported by [Tamachkiarow and Flemming \(2003\)](#). This probe applies light from below to the surface of its head and detects how much is scattered back from any deposits. It can detect bacterial colonization above 10⁵ cells per cm. This technique is not suitable for thick biofilms.

Another technique uses heat transfer monitor ([Janknecht and Melo, 2003](#)). Actually, the buildup of a biofilm on a surface imparts an additional resistance to heat transfer through the system that can be determined if the heat flux and the wall and fluid temperatures are known. Heat transfer monitor is composed of two flow channels: one for the test fluid (the one causing biofilm

formation) and the other for the auxiliary fluid (the heat source). A flat wall (metal or other material) separates the two flow channels. One thermocouple is immersed in the test liquid and two are imbedded in the wall in the same radial position. Thus, local heat transfer coefficients can be obtained along the surface. Pressure drop is also determined to take into account the effect of biofilm roughness on the convective heat transfer coefficient of the test liquid. Some monitors use an electrical resistance as a heat source instead of the hot fluid: if the heat flux provided by the electrical resistance is known, only one thermocouple is needed in the wall.

Pressure drop measurements are often used because of their simplicity and low cost. When a biofilm layer gets attached to the wall of a smooth pipe, it increases the roughness of the surface in contact with the liquid and reduces the cross-sectional area of the flow passage. These effects can be determined by measuring the pressure drop of the liquid through the pipe. The first colonies of micro-organisms that stick on the surface cause an increase in the friction factor because the surface becomes rougher. Increased pressure drop can also be due to a reduced channel cross-section, to biofilm oscillations, and to the presence of filaments on the biofilm–liquid interface. Attachment of microbial colonies of 10 μm on the surface would result in increased friction factor resulting in the pressure drop by 10% to 15%. [Characklis et al. \(1990\)](#) showed significant changes in the friction factor resulting from biofilms only after a critical thickness of about 30–35 μm was reached.

A new, affordable biofilm monitoring instrument has been developed that can detect the early formation of a biofilm growth in a water system and therefore be used in the fight against biofilm formation (www.processinstruments.com.au/.../Biofilm-Monitor-BioSense-EN-USB3). The BioSense biofilm monitoring sensors are an established technology and monitor a water system and provide an alarm when conditions allow for the growth of a biofilm. Biofilm grows preferentially on the BioSense sensor, which is encouraged by electrical stimulation. The biofilm can then be detected and remedial measures taken to destroy and remove it. The BioSense can be used to automatically instigate or change chemical dosing regimes and also to alert operators to take the appropriate corrective action. The BioSense is inexpensive, easy to maintain/operate, and can be an essential tool in monitoring the efficiency of biocide dosing regimes in many applications, including cooling towers, hospitals, large leisure facilities, swimming pools, and spas.

All living organisms leave tracks in their environment in the shape of substances produced by their metabolism. In cases where these substances are characteristic and detectable, they may be used to monitor the occurrence of the corresponding organisms. Although this procedure does not inherently discriminate between biofilms and suspended biomass, the presence of the latter may be excluded or neglected under certain conditions and the results solely referred to biofilm activity. The metabolic products in question may be liquid or gas.

Computerized image analysis has been used in several ways in the field of biofilm monitoring. Determining the portion of white and black pixels on a picture taken from a water

container's illuminated window is probably the simplest one. In more complex methods, especially designed and programmed software, can actually recognize individual cells and biofilm structures (Heydorn et al., 2000; Xavier et al., 2000, 2003).

Most of the on-line biofilm monitoring methods are based on the detection of visible, infrared, and ultraviolet light and also the radiofrequencies, all of which may be physically summarized as electromagnetic radiation signals. Among the radiation signals used for detecting the biofilms, bioluminescence signals are the only ones spontaneously produced by the biomass without requiring any input of external energy. These signals consist of light, which certain types of micro-organisms are capable of actively producing. The energy that excites the electrons in these cases is made available by an enzymatic reaction incorporated in the metabolisms. These weak light signals can be used to detect the presence of such micro-organisms. This active light emission is a function of the respective organisms' metabolic activity, which depends on several environmental factors. For that reason, bioluminescence sometimes enables the direct monitoring of such factors. On the other hand, quantitative determination of biomass by bioluminescence is only possible under favorable conditions (Angell et al., 1993). The validity of bioluminescence detection for bulk monitoring of biofilm under nonlaboratory conditions is limited.

The first step in selecting the best suited on-line biofilm monitoring method should be to find out exactly which kind of detection limit is required and which features are of interest. If the major consideration is the hindrance of flow or plugging or heat transfer of installations by biofilms, their internal features might be ignored and some representative tubing or small models of heat exchangers can be used to directly get the essential information. Instead of that, the presence and thickness of deposits including biofilms can be checked by optical methods, quartz crystal microbalances, or electrical impedance and capacitance given that the deposit can attain a certain thickness before being detected. If certain metabolic activities are important, one might concentrate on their determination either directly by means of the respective metabolic products or indirectly by bioluminescence, spectroscopy, fluorometry, attenuated total internal reflection, or electrochemical techniques. Nuclear magnetic resonance and photoacoustic spectroscopy might be taken into consideration if both the physical structure and chemical properties are of interest. For the monitoring of relatively thin biofilms, quartz crystal microbalances may be considered and also the fiber optical devices and attenuated total internal reflection depending on the biofilms' optical properties. For a very high resolution down to individual cells, one might actually consider using microscopic techniques and computerized image analysis if local conditions allow. Virtually all the techniques reported are noninvasive and allow continuous monitoring of biofilm formation. Automatic data collection is easy to put in to practice. It allows the setup of real-time, on-line methods for preventing the formation of biofilm or for cleaning the fouled surfaces that occur very frequent in the industry and present challenging opportunities for the development of expert system control techniques that are based on artificial intelligence concepts.

In general, not much information is available on the use of on-line biofilm monitoring techniques for biocide evaluations in defined laboratory conditions. This approach could be useful in understanding the mechanisms of biofilm accumulation, removal, and inhibition, and could lead to the development of new biocides. [Ludensky \(1998\)](#) reported a novel on-line, real-time, nondestructive continuous-flow system for biocide testing on industrial biofilms. This laboratory system was found to be capable of monitoring changes in growth, accumulation, and respiratory activity of biofilms in response to biocidal treatment. The system incorporates a fouling monitor for continuous measuring of the rate of biofilm accumulation (heat transfer resistance), a sensor for monitoring of microbial activity (oxygen meter for monitoring the rate of biofilm respiratory activity), and subsystems necessary for microbial life support and control of operation parameters. This technique demonstrated the capability to detect and record, in real time, the impact of the biocide treatment on biofilm growth.

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*The Control of Microbiological Problems**

Methods for controlling the microbiological problems include control of the contamination sources and control of microbiological populations (Blanco et al., 1996, 1997; Baurich et al., 1998; Bendt, 1971; Bennett, 1985). The treatment of fresh water and additives, control of the residence time in the storage tanks, and control of the stagnant flow areas should be done to control the contamination sources and the microbiological populations. Systematic maintenance and cleaning of the system by using different chemicals or treatments to reduce the formation of deposits and to eliminate the already formed deposits is required. A microbiological control program requires a good knowledge of the system and the main sources of contamination. Once the sources of contamination have been reduced, the paper manufacturer can control the microbiological populations of the system. Good housekeeping and regular inspection of all areas, effective boilouts, and regularly scheduled washup reduces slime development. To reduce the number of maintenance shutdowns in the paper machines, chemical products are introduced into the system to get rid of the microorganisms by preventing their growth or by reducing the harmful effects they produce. The chemical products most commonly used are dispersants and biocides. Other control methods are the enzymatic degradation of microbiological deposits and controlling the population by limiting nutrients (Gould, 1992, 2001; Geller, 1996; Blanco et al., 1997, 2011; Torres et al., 2011, 2012; Kanto Öqvist, 2008; Kiuru, 2011; Ullan, 2011). Joyce et al. (1980) reported that the operation of most paper mills would be virtually impossible without using chemicals to control slime formation. The desirable characteristics of slimicides, which include high toxicity and slow biodegradability, attracted regulatory attention. There is a need for green biocides derived from natural products that would be easily biodegradable. The desire for safe biocides that are green to the environment is a policy concern in the United States, Canada, and Europe. The strict US Environmental Protection Agency (USEPA) regulations may result in the removal of a large number of biocidal products that are incompatible with the environment (Treskonova and Wingenfeld, 2001).

8.1 Good Housekeeping

Good housekeeping is extremely important to deal with microbial problems (Kiuru, 2011). Thorough control of the whole-plant operations, including regular and sufficient cleaning and precise control of the delays in the use of easily spoiled materials, is of great importance

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(Blanco, 2003). Some conceptual tools to make running practices systematic and more controllable include for example good manufacturing practice and hazard analysis critical control points analysis. Basically these systems were developed for other purposes, but their principles and those of other quality-system-based approaches, can contribute to better process hygiene. This is because control of microorganisms is connected to the overall operational practices in the mill. One should also remember that the design of the equipment in mill will have important consequences in terms of microbiology (Blanco et al., 1996). Good housekeeping includes:

- Controlling the contaminants entering the process
- Performing regular boilouts of pipelines and tanks

It is much easier and significantly less expensive to keep a clean system clean than to stop a dirty system from getting dirtier. Even the best biocide program will be stressed to maintain microbial control of the process without the execution of good housekeeping practices. According to Woodward (2009), basic housekeeping starts with the standard “first in, first out” rule. To avoid long storage times, this should be basic rule for all the containers, particularly for the chemicals because they might be stored by the machine for weeks. The hoses should also be washed when filling the tanks and containers. Other good housekeeping practices include: cleaning tanks on a routine basis, sufficient agitation and recirculation, and at least annual system-wide boilouts. If the time and temperature in boilouts are limited, using some special chemicals or enzymes could be taken into consideration (Woodward, 2009). To some extent, good housekeeping can also be automated (Kiuru, 2011). On-line cleaning can be applied to essentially any position in the papermaking process. An electric-driven water pump can be used that uses the cleanest return water available from the process. The commodity items are the spray nozzles and the joint piping connections permitting ease of piping travel through elevation and direction changes to continuously clean, for example, the entire wire channel with a single flow. Parks (2007) has reported that this type of automated mechanical cleaning

- Reduces manual cleaning
- Reduces paper quality issues like spots and tears
- Reduces need for unexplained, unplanned refeed and restart exercises
- Reduces waste amount
- Improves paper quality
- Reduces biocide usage

8.2 Boilouts

Boilouts are widely used in the paper industry, particularly in fine paper mills (Lindvall, 2000; Kiuru, 2011). They are very effective at cleaning the internals, piping and headbox of the short whitewater circuit. Boilout emphasizes the use of high temperatures to clean

papermaking equipment. Steam is mostly used for the heating of solutions used for washups of the short loop of paper machine systems, but temperatures are normally in the range of 60–71 °C (Guillory, 1998; Guillory and Towery, 1998). Boilout involves treatment with an alkaline detergent solution, having a pH in the range of 10–13. But acidic solutions may also be used depending on the nature of the deposited materials. By using enzymes capable of breaking down the binder materials in a variety of deposits, the use of high or low pH solutions can be avoided (Anstey et al., 1998a,b). Paper mills periodically shut down operations and clean the wetted surfaces of a paper machine, usually with an emphasis on the short-loop recirculation system and wet-press section (Glazer, 1991; Guillory, 1998; Guillory and Towery, 1998). Papermaking efficiency and product quality often suffer if too much time elapses between such cleanups (Stitt, 1997). Schwamberger and Wormsbaker (2006) report the importance of well-planned boilouts and the advantages they offer. The boilout should include careful washing of the whole system and removal of any deposits or biofilms. The chemistry should be optimized during the boilout for optimal washing and successful startup. Careful washing without considering the chemical interactions might cause many serious problems, such as excessive foaming in startup and wrong pH levels. Boilouts can significantly increase the runnability and also improve the product quality (Schwamberger and Wormsbaker, 2006).

8.3 Biocides

Biocides are products used to control the growth of microbes and have become a necessary part of paper production as the modern process and the water supply conditions in most plants greatly promote the growth of microorganisms. Various biocides are used in the paper industry (Kanto Öqvist, 2008; Kiuru, 2011; Ullan, 2011; Blanco et al., 1997, 2002; Brattka, 1992; Bunnage and Schenker, 1995; Camp, 1989; Carvalho, 1978; Cloete and Brozel, 1991; Cloete and Gray, 1985; Eriksson et al., 1995; Farkas, 1990; Giatti, 1993; Goldstein, 1983; Haack et al., 1997; Himpler, 2001; Hootmann, 2002; Lindvall, 1998b; Lustenberger and Deuber, 1991; Mobius et al., 1986; Nelson, 1982; Paulus, 1993; Purvis and Tomlin, 1991; Rantakokko et al., 1994; Scharschmied, 1975; Schirch et al., 1993; Stomps, 1995; Van Haute, 2000; Weir et al., 1981; Kanto Öqvist, 2008; Kiuru, 2011; Kulkarni et al., 2003; Ullan, 2011; Lazonby, 1997; Barnes, 1984; Bigotte, 1979; Blanchard et al., 1987; Burka, 1993). Biocides act either by killing the microorganisms, which are known as a biocidal effect, or by inhibiting the growth of microorganisms, which is known as a biostatic effect. Properties an ideal biocide should contain are shown in Table 8.1. There are no biocides that can meet all the requirements, and none of the biocides is suitable for all applications. According to Edwards (1996), the selection of biocides must always be made site specifically. The essential parts of a successful biocide program are proper estimation of reduction in the microbial count and in the number of fungi.

Table 8.1: Properties of an ideal biocide

Performance
Be applicable over a wide range of operating conditions, such as pH and temperature Not interfere with other paper mill additives Have a broad spectrum of activity toward microbes Be efficient and fast-acting Be environmentally friendly and nontoxic (no organic solvents, heavy metals, dioxins or furans) and also safe for the operator Be low cost and easy to handle Be cost competitive
Composition
Free of organic solvents No smell Liquid (easy to handle)
Toxicity
High LD50 (i.e., low toxicity with respect to handling) High LC50 (i.e., low toxicity with respect to effect on aquatic life) Nonirritant No influence on biological waste water treatment plants Easily degradable

Based on Kiuru (2011).

Classification of industrial biocides based on their chemical structures is quite difficult (Allen, 2007). For industrial use, biocides are usually divided into two groups:

Oxidizing biocides

Oxidizing biocides are fast-killing and less costly in comparison to nonoxidizing biocides. The use of oxidizing biocides has continued to increase. An oxidizing biocide attacks microorganisms by oxidizing (an electron transfer reaction) the cell structure, disrupting nutrients from passing across the cell wall.

Nonoxidizing biocides

Nonoxidizing biocides work through various processes. These biocides interfere with reproduction, stop the respiration process, or break the cell wall. They are generally shot-fed to achieve a high enough concentration for a long enough period to kill the bacteria, algae, or fungi. Kill time generally requires several hours up to a day. In some cases, nonoxidizing biocides are found to be more effective and convenient than oxidizing biocides; therefore, both biocides are used together in many conditions such as cooling water systems. Selection of a nonoxidizing biocide depends upon many factors such as water pH, retention time, efficacy against various bacteria, fungus, and algae, biodegradability, toxicity, and compatibility with the other chemistry.

In some case, biocides have been classified on group basis and include a miscellaneous group that do not fit in any major class; sometimes biocides are also classified on the basis of their mode of action. This can be organized based on the target region of the microorganism affected by biocide action.

The types of biocides being used in the papermaking process is heavily regulated (Karsa and David, 2002). They are commonly known as slimicides and are used to reduce the buildup of slime deposits within the water phase of the process. Many changes have taken place over the past 2 decades in the paper industry. Most mills have now changed from acidic sizing processing to alkaline sizing and most of the mills have closed up their processing. In these mills, the water is now recycled within the plant and not discharged. Because of this, microbial contamination has shifted in favor of bacterial strains (because of alkaline conditions) and also increased because of the continuous reintroduction of these species and food sources back into the process. Proper use of biocides and also good plant hygiene procedures are critical for maintaining effective production and low microbiological population to prevent paper failure. The types of biocides commonly used in the papermaking process are shown in Table 8.2. The types of biocides used in the coating and additives for the papermaking process differ from the rest of the pulp and paper industry. These require longer lasting, sometimes up to 1 year's protection in storage tanks, and hence fall into the category of preservatives. The types of preservatives that are commonly encountered in the paper industry are thion, glutaraldehyde, 1,2-benzo-isothiazolin-3-one Bronopol, 1,2-dibromo-2,4-dicyanobutane, and 4,5-chloro-2-methy-4-isothiazolin-3-one.

The mechanisms of action of biocides can be divided into four broad categories: oxidants, electrophiles, lytic, and protonophores (Chapman, 2003). The oxidants—chlorine and peroxides—work directly via radical-mediated reactions to oxidize organic material (Schaechter and Lederberg, 2004; Clapp et al., 1994; Chapman, 2003; Dukan and Touati, 1996). The electrophilic agents include organic biocides such as formaldehyde and isothiazolones and inorganic ions such as silver, copper, and mercury. These biocides react covalently with cellular nucleophiles to inactivate enzymes (Collier et al., 1990; Slawson et al., 1992). They initiate the formation of intracellular free radicals that are responsible for their lethal action (Kimura and Nishioka, 1997; Thurman and Gerba, 1988). Cationic membrane-active biocides such as chlorhexidine and quaternary ammonium compounds and alcohols such as phenoxyethanol destabilize membranes, which result in rapid cell lysis (Broxton et al., 1983; Chawner and Gilbert, 1989; Gilbert et al., 1977). Weak acids, such as sorbic and benzoic acids, interfere with the ability of the cell membrane to maintain a proper pH balance that results in the accumulation of anions and cations inside the cell. Eklund (1985) reports that the inhibition of cell growth by preservatives is the result of different actions: disruption of membrane, inhibition of metabolic reactions, stress on intracellular pH, and the accumulation of toxic anions. Pyrithione is also classified as a weak protonophore (Ermolayeva and Sanders, 1995).

Biocides, both oxidizing and nonoxidizing, when applied properly can be effective in overall biofilm control. The oxidizing microbicides, such as chlorine, bromine, chlorine dioxide, peracetic acid, and ozone, can be extremely effective in destroying both the extracellular polymeric

Table 8.2A: Oxidizing biocides used today in paper industry

Oxidizing biocides
Ammonium bromide
Ammonium sulfate
Chlorine gas
Chlorine dioxide
Hydrogen peroxide
Halogenated alkylhydantoin
Peracetic acid
Sodium hypochlorite
Sodium bromide

Based on McCoy (1983), Schrijver and Wirth (2007), Paulus (1993), Kitis (2004), Simons and da Silva (2005), Heitz et al. (1996), and Kanto Öqvist (2008).

Table 8.2B: Nonoxidizing biocides used today in paper industry

Nonoxidizing biocides
1,2-Benzisothiazolin-3-on
2-Bromo-2-nitropropane-1,3-diol
5-Chloro-2-methyl-4- isothiazolin-3-on
Diethyldithiocarbamate
N,N-Dimethyl-N,N-didecyl ammonium chloride, benzalkonium chloride
2,2-Dibromo-3- nitrilopropionamide
Glutaraldehyde
Methylene bithiocyanate
2-Methyl-4-isothiazolin-3-on
Poly(hexamethylene biguanide) hydrochloride
Tetra-(hydroxymethyl)- phosphonium sulfate

Based on McCoy (1983), Schrijver and Wirth (2007), Paulus (1993), Kitis (2004), Simons and da Silva (2005), Heitz et al. (1996), and Kanto Öqvist (2008).

substances (EPS) and the bacterial cells. When using oxidizing microbicides, one must be sure to obtain a sufficient residual long enough to effectively oxidize the biofilm. Unfortunately, there are mills that are overly concerned with the corrosive nature of the oxidizing microbicides and fail to apply the needed residual oxidant required to control biofilm. Low residual oxidant levels are able to significantly reduce the planktonic counts but may not be sufficient to control biofilm. The level of oxidant and duration required is found to vary from system to system. Low-level free residual oxidant will kill planktonic bacteria, yeast, mold, and protozoa. However, medium to high dosages of free residual oxidant are needed to oxidize biofilms. Algal mats particularly require high dosages of free residual oxidant. It is generally more effective to maintain a higher residual for a duration of several hours than it is to continuously maintain a low residual. Continuous low-level feed may not achieve an oxidant concentration sufficient to oxidize the polysaccharide and expose the bacteria to the oxidant. Another misunderstanding is with the use of chlorine at alkaline pH (>8.0). It is often thought that chlorine is ineffective for controlling microorganisms at elevated

pH. This is not completely true. Surely, the hypohalous acid form of chlorine (HOCl) is more effective at killing cells than the hypohalite form (OCl⁻). However, the hypohalite is actually very effective at oxidizing the extracellular polysaccharide and the proteinaceous attachment structures. Therefore, using chlorine in alkaline cooling waters can still be tremendously effective when applied properly. This is especially true when combining chlorine with bromine or with a compatible nonoxidizing microbicide such as a polyquat. When this is done, one achieves both oxidation of the extracellular material and sufficient kill of the microorganisms. Bromine compounds, such as sodium bromide (NaBr), used to generate free residual hypobromous acid (HOBr) and organobromine biocides, are very effective oxidizing biocides. Bromine is extremely lethal to microbes, and the kinetics of the kill reactions is very quick. Certain nonoxidizing microbicides are also effective in controlling biofilm. Effective control is greatly dependent on the concentration of the product feed, frequency of addition, dosage fed, and resistance of the incumbent population to the product fed. Control cannot generally be achieved by once-a-week additions as is common in “full-service” applications. Typical application for effective control may include a slug addition of product two to five times a week. As with oxidizing microbicides, frequency and dosage will depend on the system conditions. It is generally most effective to alternate nonoxidizing microbicides at every addition to ensure broad spectrum control. Most nonoxidizing microbicides will have little effect in destroying the extracellular polysaccharide found in the biofilm. However, many of these microbicides may be able to penetrate and kill bacteria found within the biofilm, resulting in decreases in the population and weaknesses in the biofilm structure. Thus using the combination of nonoxidizing and oxidizing microbicides is a very effective method of controlling biofilm. When using a nonoxidizing microbicide in combination with an oxidizing agent, there should be a slight to no residual oxidant concentration present in the system at the time of addition. Sufficient time should be given for the nonoxidizing microbicide to work before resuming oxidant feed unless an oxidant compatible microbicide is being used. Using combination biocides has proven very successful in killing unwanted bacterial species. Application of synergistic biocides, in particular, can give improved biocide performance against harmful bacteria.

Biocides show different mechanisms of antimicrobial activity. [Paulus \(1993\)](#) has reported that glutaraldehyde reacts with amino and thiol groups in proteins, causing irreversible cross-links in the cellular constituents. Glutaraldehyde and oxidizing biocides are also effective against bacterial spores ([Paulus, 1993](#)). [Maillard \(2002\)](#) has reported that in gram-negative bacteria glutaraldehyde interacts principally with outer components of the cells, particularly lipoproteins. High degree of cross-linking means that the cells are unable to perform their important functions, resulting in a bactericidal effect. Methylene bithiocyanate (MBT) chelates Fe³⁺ ions essential for the microbial growth ([McCoy, 1983](#)). BCDMH (1-bromo-3-chloro-5,5-dimethylhydantoin) is not found to be biologically active as such, but upon hydrolysis it yields hypobromic and hypochloric acids ([Kemira Chemicals Oy, 2003](#)). Isothiazolones (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a mixture) and 2,2-dibromo-3-nitrilopropionamide (DBNPA) are electrophilic active compounds. These react with cytoplasmic constituents such as thiol groups of proteins, and inhibit cellular metabolism ([Paulus, 1993](#)). Bronopol

(2-bromo-2-nitro-propane-1,3-diol) also contains an active halogen group, but can also release formaldehyde (Paulus, 1993). Dazomet (3,5-dimethyl-1,3,5-2H-tetrahydrothiadiazine-2-tion) is rapidly hydrolyzed in water to methylene isothiocyanate (Kemira Chemicals Oy, 2003), but also releases formaldehyde (Paulus, 1993). Biocides are usually toxic with low biological selectivity. Some biocides such as DBNPA, isothiazolone mixtures, glutaraldehyde, and MBT are also sensitizing (Kemira Chemicals Oy, 2003; Paulus, 1993; Pirttijärvi, 2000). Many of the presently used biocides such as peracetic acid, hydrogen peroxide, BCDMH, DBNPA, glutaraldehyde, or isothiazolone mixtures are reactive molecules that are quickly biodegraded to nontoxic molecules and so are not harmful for the biological wastewater treatment processes. These are also not persistent in the environment (Kemira Chemicals Oy, 2003; Paulus, 1993).

Bleached pulp grades very often involve a combination of treatments with oxidizing biocides, supplemented by toxic organic biocides. It is recommended to treat each of the incoming streams, including the freshwater, filler slurries, chemical additive, and make downstreams. More attention should be given to the starch preparation area because starch is a very good food for the growth of slime. The level of oxidizing agent has to be checked at an adequately low level that there are no problems with the bleaching of dyes or decomposition of starch, etc. A residual of 1 ppm of active oxidizing agent in the paper machine system can be considered a possible starting level. Hydrogen peroxide, when used as a biocide, acts slower but the effect is long-lasting. So, it should be controlled at a higher level of residual activity in the system. The selection of toxic organic biocides can be made based on the temperature of the system and on the relative needs to control bacterial or fungal growth. It is common practice to use the toxic biocide on and off over periods from several minutes to several hours. By this means, a required threshold of activity can be reached and also the cost of the chemicals can be reduced. Such practices should be checked to ensure they do not cause excessive savings in first-pass retention or other problems. Some biocides contain anionic dispersants that interfere with retention. The residual level of oxidizing chemicals is mostly estimated by measuring the redox potential of the furnish. This is done with a platinum(Pt) electrode relative to a standard reference electrode. The effectiveness of a biocide program is best assessed with a combination of measurements which include.

1. Petri-dish cultures of water
2. Tests for the presence of biological deposits as surfaces
3. Slipperiness of wetted surfaces
4. Level of smells within the facility

By the well-organized use of slimicides and preservatives, in many mills working with largely closed water circuits and continuous production of coated papers and boards has been made possible without any problem. There is no single preparation that can solve all the preservation problems occurring in the paper industry as different types of microorganisms have varying degree of resistance to biocides. Biocides should have high activity and cost-effectiveness. The properties demanded of biocides vary according to their specific field of application. Biocides should be used in concentrations that do not upset the papermaking processes, even if they are

added in huge doses. The products should go well together with the many auxiliaries used in papermaking. In the paper used for food packaging application, no substantial amount of biocides should be present in the final product and should possess low ecotoxicity. Filler suspensions and sizes and active ingredients for the antimicrobial finish of paper and board and preservatives for coating mixes, have to meet strict requirements with regard to the absence of odor and color, compatibility, and physiological harmlessness in their use concentrations.

8.3.1 Chlorine

Chlorine is the most widely used disinfectant in public and industrial water supplies, wastewaters, and has many household applications (Kiuru, 2011). Chlorine has been used as a disinfectant since 1846. Despite USEPA regulations to limit chlorine discharge because of toxicity and carcinogen concerns, chlorine continues to be a popular choice of biocide because it is both effective and economical.

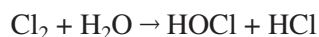
Common forms of chlorine compounds are:

- Chlorine gas
- Calcium hypochlorite
- Sodium hypochlorite

Commercial sodium hypochlorite products may contain about 15% free available chlorine. Stability of a sodium hypochlorite solution is affected by concentration, light, pH, and temperature (Casson and Bess, 2003). Factors that negatively affect the strength of sodium hypochlorite solution are:

- High temperature
- Increased hypochlorite concentration
- Storage time

The pH in liquid hypochlorite products vary between 11 and 13. In basic solution, hypochlorite anion decomposes to produce chlorate anion, which is toxic. Transition metals catalyze the decomposition of hypochlorite. Storage conditions and handling of hypochlorite stock are very important for prevention of the losses during the storage period. White (1999) has reported that the most stable hypochlorite solutions are those of low concentrations (10%), with pH of 11, with metal contents of less than 0.5 mg/L, stored in darkness and at cool temperature. Chlorine gas reacts with water to form hypochlorous and hydrochloric acids that lead to reduced pH of the water. The latter determines the biocidal activity. This process takes place according to the following reaction:



Hydrochlorous acid is responsible for the oxidation reactions with the cytoplasm of microorganisms after diffusion through the cell walls. Chlorine that disturbs the production of adenosine triphosphate (ATP), which is an essential compound for the respiration of microorganisms. The bacteria that are present in the water will die as a result of being breathed in,

breathing problems are caused by the activity of the chlorine. The amount of chlorine that needs to be added for the control of bacterial growth is determined by the pH. The higher the pH, the more chlorine is needed to kill the unwanted bacteria in a water system. When the pH values are within a range of 8–9, 0.4 ppm of chlorine must be added. When the pH values are within a range of 9–10, 0.8 ppm of chlorine must be added.

Hypochlorous acid generated by hydrolysis of hypochlorite is the active agent of sodium hypochlorite and has a stronger bactericidal effect than hypochlorite anion. It is uncharged and small and so easily penetrates the bacterial cell membrane. Most of the biocidal activity is provided by hypochlorous acid at pH lower than 7 and higher than pH 8.0, biocidal efficacy is appreciably reduced because of production of hypochlorite anion. [Deborde and Gunten \(2008\)](#) report that the best biocidal activity of hypochlorite is between 6.5 and 7.5. Hypochlorite is less effective than chlorine.

8.3.2 Bromine

Recent environmental restrictions on the usage of chlorine and new alkaline-based chemical treatment programs have increased the application of bromine-based biocides. For effective microbiological control, bromine is always fed with chlorine, either as two separate products or synthesized as a bromo-chloro compound. Hypochlorous acid is required to oxidize the NaBr component and form the biocidal species HOBr. The biocidal efficiency of bromine is similar to that of chlorine ([Kiuru, 2011](#)). Bromine is added as a bromide salt and produced by the reaction with chlorine. NaBr must be used together with an activating agent such as chlorine gas, hypochlorite, or ozone because it is not a biocide itself. [Elsmore \(1995\)](#) reports that at pH 8.5, hypobromite has higher biocidal efficiency in comparison to hypochlorite when used at equal concentrations. Several commercially available forms of bromine are:

- Bromine chloride (BrCl)
- Bromine gas
- BCDMH and other brominated hydantoin
- NaBr or isocyanurate/sodium bromide blends
- DBNPA and stabilized bromine products

The most common compounds used in cooling water are BCDMH and mixtures of sodium bromide with sodium hypochlorite or chlorine gas.

Similar to chlorine, bromine hydrolyzes in water and produces HOBr, which has the same oxidizing power as HOCl. HOBr dissociates to form H^+ and OBr^- , but the reaction takes place at a higher pH than chlorine. A bromine solution with a pH of 8.5 will contain close to 60% HOBr, whereas a chlorine solution at the same pH would yield 10% HOCl. In many cases, a smaller dose of bromine will obtain the same microbiological control as using chlorine in a cooling tower system. Another advantage of bromine is that it reacts with

ammonia and other nitrogen compounds to form bromamines that, unlike chloramines, are effective biocides. Bromine is also less corrosive than chlorine to copper alloys. Bromine reacts with iron, manganese, sulfur, and organic matter. Heat and sunlight contribute to bromine demand, but there is less stripping because of lower volatility than chlorine. Its toxicity to aquatic life and possible formation of carcinogens is similar to chlorine and has therefore led to USEPA discharge regulations. Bromine residual can be analyzed using the same N,N'-diethyl-*p*-phenylene-diamine colorimetric method and reagents as chlorine testing as long as other oxidants are absent. Like chlorine, bromine testing should be done at the time of sampling. If a spectrophotometer does not have a bromine program, the result given on the chlorine program can be multiplied by 2.25 to obtain a bromine concentration. It is usually unnecessary to test for free bromine residuals because most combined forms of bromine, such as bromamines, are just as effective as free bromine. If testing for free bromine is required, it should be noted that full color development by the N,N'-diethyl-*p*-phenylene-diamine method will take 2–3 min for stabilized bromine products instead of the directed 30 s.

Hercules Pulp and Paper Division has launched Spectrum® Ammonium Bromide Technology that efficiently controls microorganisms in alkaline systems without the adverse side effects associated with strong oxidizing biocides (Davis and Casni, 2003). This biocide degrades into inert compounds before effluent discharge. It is produced on the site by mixing an ammonium bromide solution with sodium hypochlorite and mill fresh water. Dedicated blending and dosing effluent ensures safe, consistent production of the biocide. Table 8.3 shows the benefits of the new ammonium bromide-based biocide. This biocide is produced onsite using designated dosing equipment. The dosing equipment blends the ammonium bromide solution with sodium

Table 8.3: Benefits of the new ammonium bromide-based biocide

<i>Extremely effective at reducing microbial populations (filamentous bacteria, unicellular bacteria, yeast and mold, and anaerobic bacteria)</i>	
	Reduced sheet breaks
	Reduced sheet defects
	Increased time between boilouts
	Reduced washups
<i>Exhibits a low oxidizing potential</i>	
	Reduced corrosion rates
	Reduced consumption of costly wet-end additives
	Does not damage felts
	Reduced halogenated organic compounds
<i>Is not consumed by organics, ammonia, or other compounds that typically act as demand on oxidizers</i>	
	Prevents oxidizer overfeed, which keeps program costs affordable
	Oxidizer residuals remain in system for longer time; improves microbiological population control
<i>Residual is easily measured by total combined chlorine</i>	
	Simple monitoring can optimize feed rates and prevent excessive program costs
<i>Degrades readily into nontoxic ions</i>	
	No negative effect on activated sludge plants

Based on Davis and Casni (2003).

Table 8.4: Benefits of ammonium bromide dosing system

<i>Programmable logic controller ensures correct formation of the biocide and monitors for problems</i>
Prevents unnecessary waste of biocide-producing chemicals
<i>System performs automatic shutdown sequence if an interruption of water flow, sodium hypochlorite, or ammonium bromide occurs</i>
Prevents unnecessary waste of biocide-producing chemicals
<i>Final product concentration is 0.25–0.50%</i>
The dilute biocide is not corrosive to skin and does not bleach clothing, which reduces worker exposure concerns
<i>The equipment flushes the feed lines with water after a dosing cycle</i>
No biocide remains in the lines when they are not in use, which reduces worker exposure concerns

Based on *Davis and Casni (2003)*.

hypochlorite and mill freshwater under required reaction conditions to assure 100% conversion of the components. The dosing equipment also strictly controls the reaction to ensure that only the new biocide is produced. The major features and benefits of the dosing system are presented in [Table 8.4](#). Commercial applications have verified the effectiveness of the new biocide at controlling microbial populations. The following case histories highlight some of the key benefits of this technology, which include reduced wet-end deposition, reduced breaks, and reduced papermaking additive usage. A mill producing printing and writing grades from 100% deinked pulp suffered from severe wet-end breaks because of microbiological deposition. To control this deposition, various biocides were dosed to multiple points as follows: sodium hypochlorite in the pulp chest and clear filtrate; HOBr in the short-loop whitewater; several organic biocides in thick-stock feed points; and a biodispersant in the silo. Even with extensive biocide usage, the machine experienced one to two costly breaks per day. An evaluation of the new biocide was recommended to alleviate this problem. Originally, biocide treatments were to be replaced by the new biocide in a stepwise fashion during the first 2 months of the evaluation. Within the first week of startup, the following benefits were observed with the new biocide:

- Total aerobic counts and bioactivity, as measured by ATP, were reduced by more than 99%.
- Alkyl ketene dimer sizing usage was reduced >15%.
- The number of breaks declined from one to two breaks per day to only one break in the entire first week of treatment.

At this point, the mill system was cleaner than any previous experience. These excellent results prompted the mill to replace the entire treatment program with the new biocide after the first week.

A machine making 950 metric tons per day of uncoated printing and writing paper was having problems controlling wet-end deposition. Its original program used an organic biocide to the silo (headbox loop) and broke chest of the machine. The amount of deposition on the machine was measured by an automatic online system based on weight of deposition on a stainless steel coupon. The new biocide replaced the organic biocide program at a similar

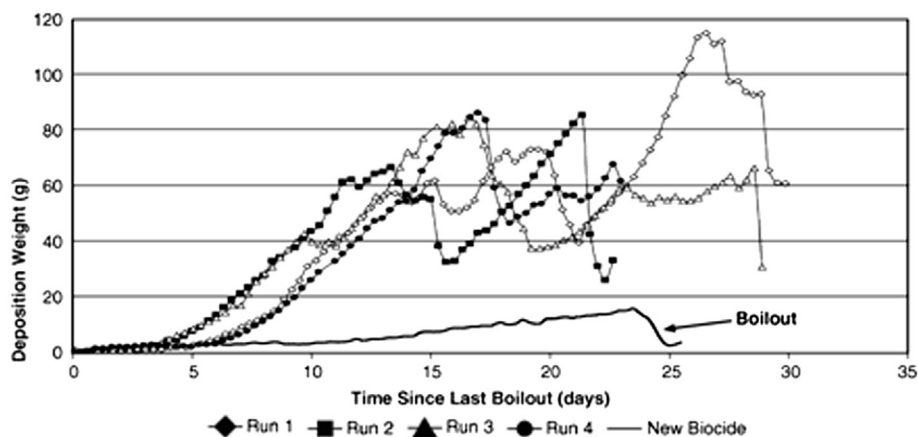


Figure 8.1

Deposition control with new biocide versus previous biocide treatment. *Davis and Casini (2003).*

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cost. Shortly after startup of the new program, the total bacterial counts dropped from 10,000,000 to 1000 colony-forming units per milliliter (cfu/mL). Furthermore, the amount of deposition, as measured by the on-line system, was reduced dramatically between boilouts (Figure 8.1). Another mill producing 200 tons per day of alkaline fine printing and writing paper was using a strong oxidizer—HOBr—to control biofilm. The HOBr was produced onsite by blending NaBr with sodium hypochlorite and then fed to the silo. An organic biocide treated the thick stock to control incoming microbiological contamination. The new biocide replaced the HOBr treatment and provided the following benefits:

- On-machine deposition was reduced.
- Sodium hypochlorite usage was reduced from 32.4 to 18.9 lb/h.
- Red and blue dye usage was reduced by 75%.

In addition to these benefits, the mill was able to completely shut off the fluorescent dye used at the size press because sheet brightness improved. Because of this success, the new biocide was then applied to an adjacent machine with the following results:

- Total bacterial counts were reduced from 230,000 to 2400 cfu/mL.
- Fluorescent dye usage was reduced at the size press by more than 80%.
- Sodium hypochlorite usage was reduced from 11.6 to 4.0 lb/h.
- Sheet brightness was increased by 2 points.

During this second evaluation, the mill realized that the original HOBr treatment had been reacting with iron in the system, which caused a brown shade in the sheet. When the treatment was switched to the new biocide, the iron was no longer being oxidized, which accounted for the increase in sheet brightness and reduced dye use.

Hootman (2002) has reported a biocide system for use in paper production. This system is effective against slime-forming and sulfate-reducing microorganisms as well as molds and algae. It is also based on the reaction of ammonium bromide and sodium hypochlorite. Unlike conventional biocides, the new system does not require limited-life stocks to be held. Instead, it is dosed into circuits at rates that can be varied for different chemical oxygen demand levels. The mill trials confirm wet-end biocide treatment improves runnability, reduces the incidence of holes and spots in finished paper, and reduces hydrogen sulfide levels. The trials covered closed loop production of unbleached liner from 100% waste paper and also mills producing high-quality bleached graphic papers from chemical pulp. The in situ biocide does not affect adsorbable organic halogen level and there is no accumulation of active biocide or of byproducts.

BromMax is patented single-feed stabilized liquid bromine biocide developed by a US company. It is based on active ingredients of NaBr and sodium hypochlorite and is used to control bacterial, algal, and fungal slime in paper mill process waters. It has the following features and benefits:

- Single-feed, preactivated solution
- Easy to handle and feed
- Enhanced stability over sodium hypochlorite bleach
- Effectiveness of bromine plus stability of nonoxidizing biocides
- Powerful biofilm removal properties
- Compatible with common scale and corrosion inhibitors

8.3.3 Chloramine

The chloramine is a mixture of monochloramine and dichloramine. It is more stable and is a weaker disinfectant than chlorine (Kiuru, 2011). Chloramine is not as reactive as chlorine with organic material in water. Therefore, it produces less disinfection byproducts. Chloramines provide better protection against regrowth of bacteria, which can be important for storage tanks and places with dead ends. But, the slow decay rates may result in higher biocide residues in the final product (Elsmore, 1995; Paulus, 2005).

Chloramines are produced by the reaction between chlorine (Cl_2) and ammonia (NH_3). Chloramines are amines that contain at least one chlorine atom, which is directly bound to nitrogen atoms. Inorganic chloramines are formed when dissolved chlorine and ammonia react. During this reaction, three different inorganic chloramines are produced:

- Monochloramine (NH_2Cl)
- Dichloramine (NHCl_2)
- Trichloramine (NCl_3).

Inorganic chloramines, free chlorine, and organic chloramines are chemically related and can change into one another easily. These compounds cannot be found in isolated form. Inorganic

chloramines are not persistent, but these compounds are more persistent than freely available chlorine compounds. Research has shown that depending on the circumstances, the half-lives of inorganic chloramines can vary from 1 min to 23 days.

Chloramines are normally produced by adding ammonia to water containing free chlorine (HOCl or OCl, depending on the pH). The ideal pH value for this reaction is 8.4. This means the water is slightly alkaline. Reaction mechanism is shown as follows:



When the reaction takes place, three kinds of inorganic chloramines can be formed. The pH value determines which kind of chloramines is formed. Trichloramines mainly form when the pH value is 3 or below. When the pH value is 7 or above, dichloramine concentrations are highest. The amounts of chlorine and ammonia in the water also influence the origination of chloramines. The chlorine/ammonia rate is ideally 6:1. During chloramine, the rate is usually 3–5:1. When ammonia concentrations are higher, more di- and trichloramines are formed. Organic chloramines can also be formed during these reactions. Organic chloramines cannot be distinguished from other chloramines, using standard chloramine analysis. Table 8.5 shows the properties of various chloramines. Figure 8.2 shows the structures of monochloramine, dichloramine, and trichloramine.

Keegan et al. (2010) did several experiments comparing vapor phase corrosion of different oxidizers using paper machine whitewater and steel plates of different grades. Table 8.6 gives the results from an experiment comparing the vapor phase corrosiveness of water, monochloramine (MCA), and monochloro-5,5-dimethylhydantoin (MCDMH), a partially halogenated hydantoin (Sweeny et al., 2002). Results in Table 8.6 show that MCA was substantially more corrosive in the vapor phase than MCDMH at similar dosage levels on the basis of total active

Table 8.5: Properties of various chloramines

Name	Molecular weight	Preferred pH value	Biocidal effect
Monochloramine NH_2Cl	52	>7	Good
Dichloramine NHCl_2	85	4–7	Tolerable
Trichloramine NCl_3	119	1–3	Average
Organic chloramines RNHCl	Varies	Unknown	Bad

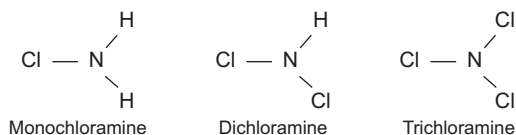


Figure 8.2

Structures of monochloramine, dichloramine, and trichloramine.

Table 8.6: Comparison of vapor phase corrosiveness of monochloramine (MCA) and monochloro-5,5-dimethylhydantoin (MCDMH) on EN10149-2 low carbon steel

Treatment	Dosage as total active chlorine (ppm)	Vapor phase corrosion of the steel coupons after 7 days	
Untreated reference	0	–	4
MCA	5	+++	21
MCDMH	5	–	7
MCA	10	++++	44
MCDMH	10	–	6

Laboratory experiment was performed with authentic paper machine water.

Based on [Keegan et al. \(2010\)](#).

Table 8.7: Halogenated hydantoins

Dichlorodimethylhydantoin	1,3-dichloro-5,5-dimethylhydantoin	$C_5H_6Cl_2N_2O_2$
Bromochlorodimethylhydantoin	1-Bromo-3-chloro-dimethylhydantoin	$C_5H_6BrCl_2N_2O_2$
Dichloroethylmethylhydantoin	1,3-dichloro-5-ethyl-5-methylhydantoin	$C_6H_8Cl_2N_2O_2$
Dibromodimethylhydantoin	1,3-dibromo-5,5-dimethylhydantoin	$C_5H_6Br_2N_2O_2$
Bromochlorodimethylhydantoin	1-bromo-3-chloro-5,5-dimethylhydantoin	$C_5H_6BrClN_2O_2$

chlorine. Tested concentrations of MCDMH did not show any significant difference from the untreated whitewater reference during the experimental period.

Kemira has applied for a patent on the dual use of MCA and MCDMH for corrosion safe microbe control on paper machines. The basis of this technique is to take the advantages of both chemistries along with advanced monitoring technique (PiBa assay) to provide the safest and economical treatment. MCA is added in broke system, save-all, and thick stock streams to lower the general activity of planktonic microbes in the system. MCDMH is added to the short circulation and press section showers. This combination is beneficial for wet-end stability, while at the same time minimizing MCA carryover to the dry section where vapor phase corrosion concerns are the highest. MCDMH is applied to control biofilm formation in a corrosion safe manner.

8.3.4 Halogen-Release Biocides

The halogen-release biocides are organic biocides. In contact with water, these biocides generate hypochlorous and/or HOBr. Hydantoins release chlorine and bromine. A few examples include: 1,3-dibromo-5,5-dimethylhydantoin, BCDMH, and 1,3-dichloro-5,5-dimethylhydantoin. [Table 8.7](#) shows various halogenated hydantoins along with their chemical formula. [Figure 8.3](#) shows the structures of various halogenated hydantoins.

Several hydantoin-based products are available for use in papermaking to control slime development ([Syke, 2006](#)). The major advantages of these compounds are their stability and

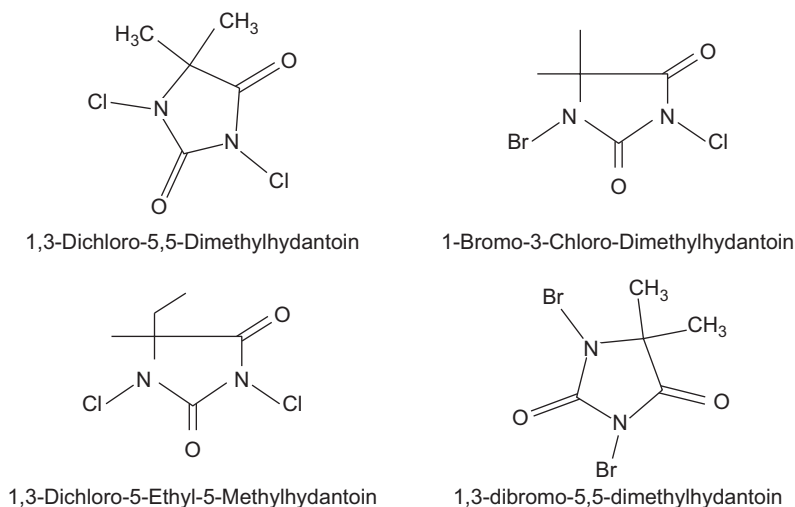


Figure 8.3
Structures of halohydantoin.

being adversely affected by organic matter even though at a lower degree than chlorine (Bloomfield and Miller, 1989). Halogenated hydantoin are used in water cooling tower disinfection, toilet bowl cleanser, and swimming pool and hot tub disinfections.

Interest in using combined halogens has been renewed and papermakers are currently using a number of combined halogen products for the control of microorganisms (Bruce, 2003). Over the past several years, a number of oxidant products have become available in the market (Bruce, 2003; Thomas, 1999). These products consist of halogens, bromine and/or chlorine, combined with an organic or inorganic carrier. One major advantage to combining the halogen is that it can often reduce the negative effect of the oxidant while maintaining its biocidal properties. The objective of combining a halogen with another molecule is to make halogen less aggressive but still biocidal. Bleach or chlorine gas with ammonia was mixed in the 1930s and 1940s to make chloramine for microbiological control in papermaking. Even though chloramine treatment was found to be more effective than chlorine, the chemistry was abandoned. The reasons for abandoning chloramine treatment were increased corrosion and increased microbiological activity resulting from the ammonia, which is a good source of nitrogen for bacteria. Later, bleach in combination with sulfamic acid (chlorosulfamate), was recommended as a biocide with low reactivity to process equipment and chemistries but the product was not successful because of the weak biocidal activity of chlorosulfamate. The various trade names often make it confusing to determine the number and nature of the products available.

Papermakers are presently using four types of chemistries:

- Hydantoin
- Sulfamate

- Ammonia/ammonium
- Isocyanurate

Hydantoin group: This group consists of bromine, chlorine, or both attached to a hydantoin molecule. Halohydantoins are not very stable in liquor form, so they are produced and sold as a solid product either in the form of powder, granules, or briquettes. Feeding this product requires the use of a powder feeder or a brominator that consists of a vessel with granules or briquettes. Water is flown through the vessel, which dissolves the product, and is sent to the process. Powder feeders work by making a slurry and delivering that slurry to the process. The product fully dissolves once in the process.

Sulfamate group: This group consists of bromine or chlorine attached to a sulfamate molecule. Halosulfamates can be produced as stable liquid products unlike the halohydantoins. Presently, only bromosulfamate is used for paper mill water treatment; chlorosulfamate is used in some cooling tower applications.

Ammonia/ammonium: This can be produced by mixing chlorine or bleach with ammonia or an ammonium salt. These two products can be premixed before application to the process water or can also be mixed in the process water. These types of haloamines cannot be produced as stable liquid products. A combination of bleach with ammonium bromide has been introduced as a way to produce a new haloamine oxidant.

Isocyanurate group: This consists of chlorine attached to an isocyanurate molecule, but NaBr may also be present, allowing formation of hypobromide. Halocyanurates are produced in solid form like halohydantoins.

Potential benefits of combined halogens are:

- Persistence
- Increased efficacy in high-oxidant demand systems
- Better slime penetration and removal
- Better compatibility with papermaking chemistries and with papermaking equipment

BCDMH is a cost-effective, fast-acting biocide. It is fully compatible with the conditions found in modern papermaking practices. It offers the paper manufacturer a very good solution to microbiological problems by beating the disadvantages of traditional nonoxidizing biocides. This product is found to be three times more effective on filamentous bacteria, which is selectively found in paper machine fresh water and slime deposits. It is a white crystalline compound slightly soluble in water having a melting point of 159–163 °C, 1,3-dichloro-5,5-dimethylhydantoin belongs to the family of imidazolone compounds (Rao et al., 2002). This compound shows low solubility in water, but part per million levels are enough to serve as a good disinfectant and bactericide because it slowly decomposes to produce free chlorine in water (Rao et al., 2002). After this process, the remaining compound (5,5-dimethylhydantoin) can be rapidly decomposed into ammonia and carbon

dioxide by light, oxygen, and microorganisms without leaving any environmentally polluting residues.

A particular combination of halogenated hydantoin has shown better efficacy than other halogen-based slimicides under both acidic and alkaline mill water conditions (Sweeny et al., 2002; Knapick et al., 2003). This combination includes 1-bromo-3-chloro-5,5-dimethylhydantoin, 1,3-dichloro-5,5-dimethylhydantoin, and 1,3-dichloro-5-ethyl-5-methylhydantoin. This combination of hydantoins is referred to as BrMEH, for bromine methylethylhydantoin. Knapick et al. (2003) found that BrMEH increases whitewater efficacy in comparison to other oxidizing alternatives. BrMEH is rapidly converted to residual nonhalogenated hydantoin and a halide ion salt. Testing has also indicated that BrMEH hydrolyzes completely in water. Other halo-hydantoins follow similar pathways, and their degradation products are of a similar low toxicity. BrMEH is easier to handle than liquid oxidative biocides and does not require as much labor. An extensive study of a mid-Atlantic tissue mill resulted in several process recommendations, including an extended BrMEH trial. These trials showed that BrMEH effectively controlled aerobic and sulfate-reducing bacteria and maintained machine cleanliness and product quality. The improved effectiveness of the hydantoins can be explained by the formation of a moderately bound chlorohydantoin species in equilibrium with biocidally active hypochlorite. This equilibrium stabilizes active chlorine in a relatively unreacted combined form, thus releasing biocidally active hypochlorite on demand. So, active halogen lifetime is increased and biocidal efficacy is enhanced. This phenomenon accounts for the superior field results of the product where other inorganic and organic oxidants have not performed well. When the binding affinity of halogen is increased, halogen activity and release is further reduced, but biocide efficacy is also reduced because the release of the active hypochlorite is greatly inhibited. Thus, hydantoins provide an optimum balance of reactivity reduction and hypochlorite release, resulting in a very effective water system biocide for the paper industry. Also, the potential for total organic halogens to find their way into the effluent is reduced because the requirement for applied halogen is reduced. The possibility that the finished paper product will be affected is greatly reduced because less oxidant is used and there is less potential for system metals to corrode. The reduction in corrosion potential decreases any potential effect on the Yankee dryer coating and allows the biocide to be used closer to the wet-end of the machine. The environmental fate of any biocide is a main consideration of any biocide program. A study of papermaking biocides gave halo-hydantoins a favorable environmental profile. This conclusion was based on the rapid detoxification of active halogen species and the low toxicity of the unhalogenated residuals.

8.3.5 Chlorine Dioxide

Chlorine dioxide is an ideal biocide. It is found to be effective in the control of microbiological growths in paper mills under conditions unfavorable to chlorine. It is particularly effective in systems having a high pH, ammonia-nitrogen contamination, persistent slime problems, or where the microbial contamination is aggravated by contamination with vegetable or mineral

oils, phenols, or other high chlorine-demand producing compounds. Unlike chlorine, chlorine dioxide does not react with organic materials to form trihalomethanes. As a broad-spectrum, oxidizing biocide, chlorine dioxide generated is effective for use in controlling microbiological growth in whitewater paper mill systems. Although chlorine dioxide is nonreactive with ammonia-nitrogen, it may oxidize some sheet additives such as wet strength resins or retention aids. Chlorine dioxide is a strong oxidizing agent and is also widely used as an odor control agent (Baker, 1981; Giatti, 1993; Nelson, 1982; Anonymous, 1990b). It consists of one chlorine atom and two oxygen atoms (Figure 8.4). It is slowly becoming an important tool in disinfection and oxidation in the world today. Physical and chemical properties of chlorine dioxide presented in Tables 8.8 and 8.9 show its amazing capabilities. Chlorine dioxide does not constitute a risk against the environment. The Alliance for Environmental

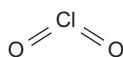


Figure 8.4

Structure of chlorine dioxide.

Table 8.8: Physical properties of chlorine dioxide

Molecular weight of 67.45
Gas at normal temperatures and pressures
Melting point of -59°C
Boiling point of 11°C
Yellowish/green and has an odor similar to that of chlorine
Denser than air and is water soluble at standard temperatures and pressures up to 2500 ppm
Explosive in air at concentrations $>10\%$
Prohibited from all form of transport, it is normally generated at the point of application
Decompose in the presence of ultraviolet, high temperatures, and high alkalinity ($>\text{pH } 12$)

Table 8.9: Chemical properties of chlorine dioxide

Chlorine dioxide does not dissociate in water
Chlorine dioxide is an oxidant with a low redox potential
Chlorine dioxide has a few specific chemical reactions
Chlorine dioxide has a very high efficacy against vegetative cells, for example, bacteria, fungi, yeasts, and molds; viruses; algae; and protozoa. It has little to no effect on human, animal and fish cells. It has been shown to have high efficacy against molluscs and acracides with unconfirmed reports suggesting some action against nematodes
The low oxidation potential of chlorine dioxide means that it can penetrate biofilm and indeed chlorine dioxide has been proven as the most effective chemical against biofilm
Chlorine dioxide is a factor lower in dosage for the same efficacy against bacteria and fungi when compared against any other standard disinfectant like chlorine, iodine, bromine, hydrogen peroxide, quaternary ammonium compounds, glutaraldehyde, and phenolic and peroxyacetic acid formulations

Technology has indicated that the “environmental risks of a modern paper mill using chlorine dioxide are insignificant.” Alliance for Environmental Technology is a group of 19 North American chemical manufacturers and forest product companies, established to promote proven and practical technologies to raise the environmental awareness.

Chlorine dioxide rapidly kills bacteria, viruses, and *Giardia*, and is also effective against *Cryptosporidium*. Chlorine dioxide also improves taste and odor, destroys sulfides, cyanides, and phenols, controls algae, and neutralizes iron and manganese ions. It is an effective biocide at concentrations as low as 0.1 ppm and over a wide pH range. It is 10 times more soluble in water than chlorine, even in cold water. Unlike iodine, chlorine dioxide has no adverse effects on thyroid function. Chlorine dioxide is widely used by municipal water treatment facilities. Chlorine dioxide is approved and recommended by USEPA as an environmentally friendly drinking water additive to replace chlorine. Chlorine dioxide has been called the “ideal” biocide for a number of reasons:

- It works against a wide variety of bacteria, yeasts, viruses, fungi, protozoa, spores, mold, mildews, and other microbes (Knapp and Bettisti, 2001).
- It exhibits rapid kill of target organisms, often in seconds.
- It is effective at low concentrations and over a wide pH range.
- It biodegrades in the environment.
- Unlike chlorine, it does not generate harmful by-products.

Chlorine dioxide works by penetrating bacteria cell walls and reacting with vital amino acids in the cytoplasm of the cell to kill the organism. The by-product of this reaction is chlorite, which is not known to pose significant environmental or human health risks.

Chlorine dioxide has a lower oxidation potential compared to ozone and chlorine. The optimal pH is between pH 6.0 and pH 10.0 and is generally more effective against microorganisms at pH above 8.0 than chlorine (Knapp and Bettisti, 2001). Chlorine dioxide is converted to chlorite, the predominant end-product (50–70%), and to chlorate and chloride in water (Baribeau et al., 2002). The major advantage of using chlorine dioxide is that it reacts less with ammonia as compared with chlorine. Though chlorine dioxide is the strong oxidizing agent, it is not very effective against established biofilms. For example, Jang et al. (2006) reported that when chlorine dioxide used at a concentration of 25 ppm, it failed to remove a biofilm thicker than 100 μm . Chlorine dioxide is used as a cellulose bleaching agent, for water disinfection at paper manufacturing plants public, and in water treatment facilities. Production of chlorine dioxide onsite can be carried out with Eka chlorine dioxide PurateR technology by Eka Chemicals Systems. Chlorine dioxide is produced from sodium chlorate, hydrogen peroxide, and sulfuric acid in Eka’s SVP-PureR generator (Koepenick, 2010). Chlorine dioxide provides broad-spectrum kill of microorganisms. Processing equipment can be kept free of slime buildups by the fast killing rates of chlorine dioxide. Slime control program using chlorine dioxide improves the quality of paper products by reducing defects

such as specks, spots, and holes in the sheet. This reduces sheet breaks and avoids the subsequent production losses. Chlorine dioxide can be used for controlling odors. Chemical spot testing for chlorine dioxide at various points in the system is easily done and can be used to make adjustments in the treatment program to make up for demand changes. This allows slime control by chemical control. Most mills alternately apply several types of biocides to avoid developing resistance by certain troublesome microorganisms to a single product. This possibility is avoided with oxidizing type of chemical. It functions well over a broad pH range. This is important for the mills that operate paper machine at different pH levels, because of the different paper grades produced or because sizing changes have raised the pH in the papermaking processes. The performance of chlorine and some nonoxidizing biocides drop off in alkaline pH environment, but chlorine dioxide does not. The effective dosages of chlorine dioxide are usually low, making chlorine dioxide programs cost competitive with other biocides. Low dosage rates can result in cost reduction for effective slime control and significantly reduce the potential harmful effects to the environment from the mill effluent water. The MD Papéis' Santista mill, located in Cubatão, São Paulo State, Brazil, decided to switch from a monochloramine-based system. The mill produces 60,000 tons per year of printing and writing grades and flexible packaging. Because monochloramine is a persistent chemical and can be harmful to waste treatment, MD Papéis Santista began looking for a way to reduce the toxicity of their effluent. Purate provided the level of treatment efficiency desired without the persistency problems in the effluent, and also helped with paper machine runnability.

Several mills are running trials in the United States and look to be fully commercialized in the near future. A simple conversion to chlorine dioxide, delivered by a compact generator, addresses these issues, and consequently eliminates persistent deposits on forming fabrics, press felts, and equipment. Foul odors, increased calcium levels, and high conductivity can also be drastically reduced. According to Jim Anderson, Purate, Eka Chemicals' "compact chlorine dioxide" is the most cost-effective slime control option for paper machines. Typically, payback is less than 6 months because Eka Chemicals takes responsibility for installing and operating the generator. The initial cost for the customer is limited to polyvinyl chloride piping, electrical and distributed control system connections, including tote bin handling and containment. [Figure 8.5](#) shows the principle of system for Eka Purate application.

The small-scale generator enables a chlorine dioxide supply from 0.5 kg/h up to 100 kg/h. This technology is widely employed for many applications throughout Europe and North America, including manganese/iron oxidation and disinfection of drinking water, microorganism control in effluent and cooling water, odor abatement in industrial water, replacement of sodium hypochlorite and chlorine in fresh water, and slime control on paper and board machines. Significant prevention of formation of slime on surfaces in the chlorine dioxide line reduced total aerobic count in the treated clear filtrate. The objective of a full-scale trial was to increase runnability on the paper machine by reducing microbiological activity, thus

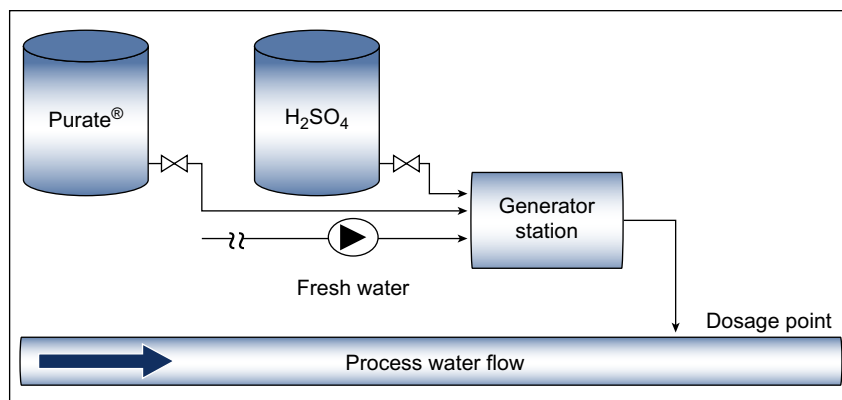


Figure 8.5

Principle of system for Eka Purate application. [Koepenick \(2006\)](#). Reproduced with permission.

reducing slime formation. To establish a clear picture of conditions in the water systems of the paper machine, a microbiological survey is always performed before startup.

One important parameter to measure is redox potential (in mV), which can be used as a control signal for chlorine dioxide dosing. Chlorine dioxide is normally added to one or several addition points in the long and short circulation systems (e.g., whitewater, clear filtrate, wire pit).

Early results of the full-scale trial demonstrated:

- Improved runnability
- A reduction in lower quality production
- Significantly reduced microbiological activity
- Significantly reduced formation of pink slime.

The results showed that chlorine dioxide could significantly limit biofilm and slime formation. [Figure 8.6](#) shows results of purate treatment with water of linerboard machine.

Because chlorine dioxide is used for potable water disinfection, it is appropriate to use this versatile disinfectant in food-grade paper applications. Food-grade paper is required to meet higher microbial standards than fine paper. Therefore, the cost of microbiological control is considerably higher than for fine paper. This is because it is difficult to inactivate bacterial spores, particularly the genus *Bacillus*, which survives the extreme temperatures of the dryers in the papermaking process. Chlorine dioxide has been found to be a very effective sporicide in food-grade paper applications ([Bendt, 1985](#); [Conkey, 1981](#)) in potable water applications ([Ridenour et al., 1949](#); [Sokolova et al., 1969](#)), and in some food processing applications ([Foegeding et al., 1986](#); [Ito and Seeger, 1980](#)). Unlike chlorine, chlorine dioxide is relatively nonreactive with most of the organics found in alkaline whitewater. As a result, a large portion of the chlorine dioxide fed will be available for disinfection. Thus the bacterial activity can be

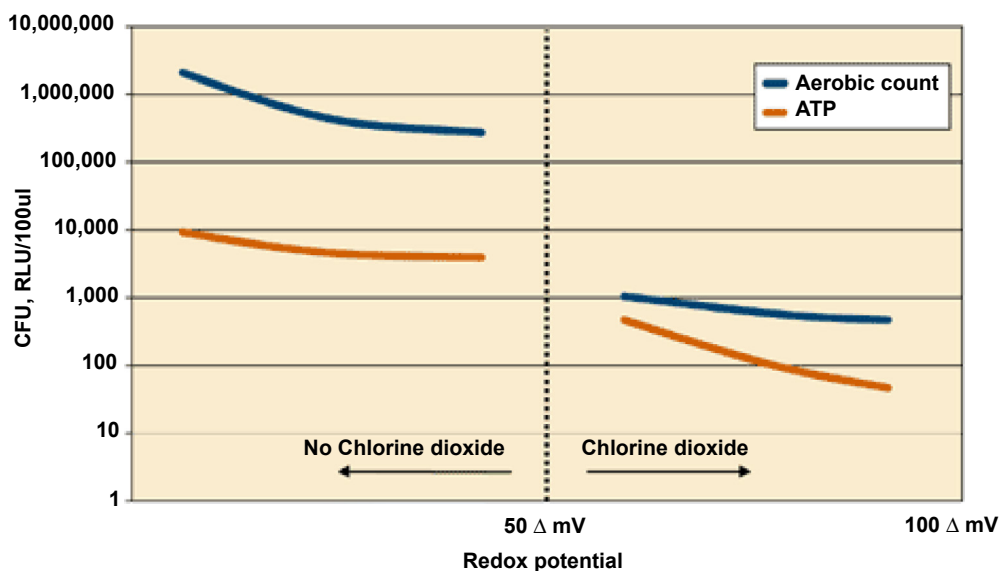


Figure 8.6

Results of purate treatment with water of linerboard machine. Aerobic count and ATP versus redox potential. *Koepenick (2006). Reproduced with permission.*

effectively reduced to almost any desired level by controlling the chlorine dioxide feed rate. A summary of the important benefits of chlorine dioxide is presented in [Table 8.10](#).

The required dosages will vary with water conditions, the severity of contamination, and the degree of control desired. For control of bacterial slime, the required chlorine dioxide residual concentrations range between 0.1 and 5.0 mg/L. Chlorine dioxide may be applied either continuously or intermittently. The typical chlorine dioxide residual concentration range is 0.1–1.0 mg/L for continuous doses, and 0.1–5.0 mg/L for intermittent doses. The minimum acceptable residual concentration of chlorine dioxide is 0.1 mg/L for a minimum 1 min of contact time.

Chlorine dioxide is a gas produced by activating sodium chlorite with an oxidizing agent or an acid source. Sodium chlorite is converted to chlorine dioxide through a chlorine dioxide generator and applied as a dilute solution. Chlorine dioxide solutions should be applied to the processing system at a point, and in a manner, which permits proper mixing and uniform distribution. The feed point should be well below the water level to prevent volatilization of the chlorine dioxide. Coincident feeding of chlorine dioxide with lime or powdered activated carbon should be avoided.

8.3.6 Hydrogen Peroxide

Hydrogen peroxide is much less effective biocide than, for example, hypochlorite. Its chemical formula is H_2O_2 and its structure is shown in [Figure 8.7](#). The bactericidal action of hydrogen peroxide is due to generation of hydroxyl radicals that oxidize thiol groups in proteins (*Russell, 1998; Denyer and Stewart, 1998*). Hydrogen peroxide produces oxygen from

Table 8.10: Benefits of chlorine dioxide

Chlorine dioxide is a very effective slime control agent.
Chlorine dioxide reacts rapidly and can be applied at a site immediately before the problem area, unlike many conventional antimicrobials, which are generally slow acting.
Chlorine dioxide remains relatively nonreactive with the vast majority of organics, reducing the dose rate necessary to achieve effective control.
Low dose rates result in typically low corrosion rates when compared to other oxidizers. In addition, minimizing or eliminating the slime layer reduces microbiologically influenced corrosion on equipment.
The chlorite ion (chlorine dioxide byproduct) keeps working as both a bacteriostat and slime control agent, even after the chlorine dioxide has reacted.
By effectively controlling slime growth, the frequency of boilouts can be reduced and the potential for unscheduled downtime because of paper breaks can be minimized.
Effectively controlling slime growth minimizes the hole count, maintaining the quality of the finished sheet.
Odors resulting from bacterial fermentation, phenols, sulfides, or mercaptans are virtually eliminated by use of chlorine dioxide.

Based on <https://final-test.oxy.com/.../SodiumChlorite/Bacterial%20Slime%20Cont>.

**Figure 8.7**

Structure of hydrogen peroxide.

solution when reacted with organic matter. It reacts with most materials including metals and so it is quickly consumed by organics and nonorganics. Some commercial products have additives that increase bactericidal action of the biocides. At low temperatures or low concentrations, hydrogen peroxide is not a powerful biocide but it exhibits a strong biostatic effect inhibiting growth of many microbiological species (Chiari et al., 1990; Rantakokko et al., 1994; Schirch et al., 1993). The biocidal preparation is found to increase with both temperature and concentration. It has been used to control anaerobic bacteria in the paper industry, as a sterilant for aseptic packaging for milk and also fruit juice containers. Hydrogen peroxide is often used in conjunction with peroxyacetic acid (PAA), existing in an equilibrium mixture designed to specific formulations to achieve the greatest effectiveness for paper industry applications. Hydrogen peroxide generates hydroxyl radicals ($\text{HO}\cdot$), which is highly reactive and responsible for the antimicrobial action. It can attack membrane lipids, DNA, and other cell components. Catalase and peroxidase are enzymes produced in respiring cells to protect the cells from damage by steady-state levels of metabolically generated hydrogen peroxide. Hydrogen peroxide is effective between pH 2 and 10 and active against spores.

8.3.7 Peroxyacetic Acid

PAA is an extremely powerful, fast-acting biocide. PAA is a clear, colorless liquid with no foaming capability. It has a strong pungent acetic acid odor (acetic acid [AA] is the principal

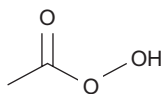
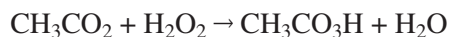


Figure 8.8
Structure of peracetic acid.

component of vinegar) and has an acidic pH of less than 2. It is soluble in water in all proportions and in polar organic solvents. However, it is slightly soluble in aromatic solvents. Peracetic acid or PAA is the peroxide of AA. [Figure 8.8](#) shows the structure of PAA. It has long been used in sewage treatment and in the sugar, dairy, and brewery industries as well as for medical sterilization for renal dialysis machines. PAA is used widely for cold sterilization and disinfection. It is also effective for both drinking and wastewater treatment ([Kitis, 2004](#); [Rossi et al., 2007](#)). PAA-based biocides are found to be effective for controlling microbial populations in papermaking process waters ([Bjorklund, 2000](#); [Maunuksela, 1995](#)). Because PAA is used rather fast and does not leave any toxic residue, it can be an attractive biocide for mills that produce food-grade paperboard. Peracetic acid is rapidly tidal at low concentrations against a broad spectrum of microorganisms, including gram-positive and gram-negative bacteria, yeasts, molds, and algae under a wide variety of conditions. It is also effective against anaerobic and spore-forming bacteria. Peracetic acid is effective at killing biofilm microorganisms at low concentrations and short contact times. Unlike a number of other biocides, the biocidal activity of peracetic acid is not affected by pH or water hardness and biocidal activity is retained even in the presence of organic matter. For these reasons, peracetic acid is well-suited as a biocide in industrial cooling water and papermaking systems. Peracetic acid is compatible with additives commonly used in these systems. Although peracetic acid is a potent biocide, it is unique in that it does not produce toxic byproducts and its decomposition products, AA, water and oxygen, are innocuous and environmentally acceptable. PAA has a broad spectrum of activity over a wide temperature range. Once reacted, it breaks down to nontoxic end-products—water, oxygen, and AA—which itself breaks down to carbon dioxide and water.

PAA is a strong oxidant and disinfectant. Its oxidation potential is larger than that of chlorine or chlorine dioxide. PAA is commercially available in the form of a quaternary equilibrium mixture containing AA, hydrogen peroxide, PAA, and water as shown by the following equation ([Kitis, 2004](#)).



Where

$\text{CH}_3\text{CO}_2\text{H}$ = acetic acid

$\text{CH}_3\text{CO}_3\text{H}$ = peracetic acid

H_2O_2 = hydrogen peroxide

The products of PAA decomposition are AA, hydrogen peroxide, oxygen, and water. There are three reactions in which PAA is consumed in an aqueous solution:

- Spontaneous decomposition
- Hydrolysis
- Transition-metal catalyzed decomposition

In Finland, the PAA mixture Desirox has been successfully used to control microbial growth in waters of the paper mill process ([Maunuksela, 1995](#)).

Although it is claimed as an oxidizing biocide, the mode of activity is not merely oxidation, as the molecule penetrates the cell wall to give a greater effect than pure oxidation. There is also no known immunity to PAA, provided sufficiently high active levels are maintained. It is nonfoaming and can reduce chemical and biological oxygen demand in effluent.

The active ingredients can be easily monitored using proprietary electro-optical measuring equipment, giving parts per million concentrations within seconds. The chemistry does have some limitations. It is found to be not much effective on organisms with thicker cell walls such as filamentous bacteria and molds. Certain system chemistries—for instance, high levels of carbonate filler—can mean that higher dosage rates are required to effect control. The control program has been used successfully in UK's Paper New Thames Mill, Kent ([Bhattacharjee and Farr, 1977](#)). There are several different commercially available PAA-based biocides. It has rapid bactericidal activity against different vegetative organisms and spores ([Baldry, 1983](#)). The bactericidal effectiveness of PAA is affected by temperature and pH ([Cords and Dychdala, 1993](#)). The presence of organic compounds adversely affects the biocidal activity of PAA. PAA is effective over a broad pH range (pH 1.0–8.0); however, the optimum antimicrobial activity occurs in acidic environment. The activity of PAA is found to decrease at pH higher than 8 ([Cords and Dychdala, 1993](#); [Sanchez-Ruiz et al., 1995](#)). AA present in high amount in PAA-based biocides may have a negative effect on the pH stability of a papermaking process. Another disadvantage associated with PAA disinfection is the increase of organic content and the potential microbial regrowth because of remaining AA ([Kitis, 2004](#)).

The sporicidal properties of peracetic acid, hydrogen peroxide, chlorine, and formaldehyde were compared by [Alasri et al. \(1993\)](#) in vitro using a dilution-neutralization micromethod. A combination of peracetic acid and hydrogen peroxide was also tested to assess their interactions. The activities of these agents, which are widely used as disinfectants, were evaluated against *Bacillus* spore isolates found on stored membranes and collection cultures. Peracetic acid and chlorine exhibited an excellent antimicrobial activity, with a destruction of 10^5 spores/mL after 5 min of contact. Generally the effects of the biocides tested were time-dependent. The sporicidal activities of hydrogen peroxide and formaldehyde were the lowest. The combination of peracetic acid and hydrogen peroxide, tested by a checkerboard micro-method, was found to be synergistic. The minimal sporicidal concentration (MSC) was

established in terms of time for each biocide. The lowest MSC values for peracetic acid, hydrogen peroxide, chlorine, and formaldehyde were:

Peracetic acid: 168–336 ppm (1–2 h of contact)

Hydrogen peroxide: 5625–11,250 ppm (5–7 h)

Chlorine: 168–336 ppm (2–3 h)

Formaldehyde: 1875–3750 ppm (5–30 min)

The MSC of a biocide combination of peracetic acid and hydrogen peroxide showed that synergy was maintained with increasing contact time and that the MSC could be reduced by two to eight times when compared with those of the biocides alone. Optimal concentrations and contact times of those chemicals that were promising *in vitro* were then tested for their ability to disinfect ultrafiltration membranes. The sporicidal activities of peroxide compounds and chlorine were confirmed and the synergism between peracetic acid and hydrogen peroxide was also maintained.

Aquabond Inc. Canada Spotless Sanitize is an effective biocide that uses the strong oxidizing properties of PAA. It is used to prevent biofilm or “slime” formation. In turn, it is a proactive odor eliminator generated by bacteria in paper mills. In addition, the effectiveness of Spotless Sanitize at low temperatures and over a wide pH range makes it an ideal bleaching agent for the pulp and paper industry. The resulting products reach and maintain their brightness goals without yellowing. Spotless Sanitize is an environmentally friendly alternative to aldehydes, bromium, organic sulfur, and quaternary ammonium biocides as well as chlorinated bleaches.

The hydrogen peroxide present reacts with the polysaccharide layer of the biofilm causing it to disrupt, at which point the peracetic acid will destroy the microorganisms present. Solvay’s initial trials regarding the application of Proxitane involved the tandem addition of an organic biocide and hydrogen peroxide. The efficiency of that system led to the elimination of all organic biocides replacing them with Proxitane. The other drawback of the single use of most types of organic biocides is the possibility of immune strains of microorganisms developing in whitewater systems that would then require a multibiocide addition. The known wide-spectrum biocidal activity of Proxitane would overcome this. There is no known immunity to peracetic acid. [Table 8.11](#) shows advantages of Proxitane.

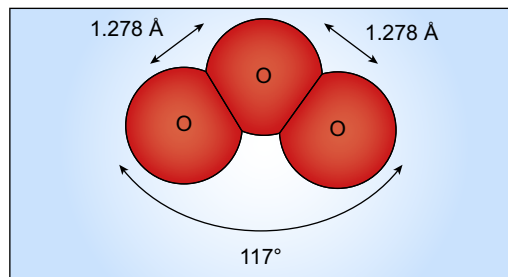
8.3.8 Ozone

Ozone (O₃) trioxygen, is a triatomic molecule, consisting of three oxygen atoms ([Figure 8.9](#)) It acts as an oxidizer, much like chlorine or bromine, improving system turbidity and removing bioslime ([Table 8.12](#)). The biocide action of ozone is a result of its reaction with the double bonds of fatty acids of the bacterial cell wall and membrane. The application of ozone results in a change in bacterial cell permeability and a leakage of cells contents into solution. The action of ozone in water is immediate and after performing its action it reverts back to oxygen. It decomposes in solution producing hydroperoxyl, hydroxyl, and superoxide

Table 8.11: Advantages of proxitane

Effective against a broad spectrum of microorganisms
Operates over a wide temperature range
Removes biofilm
Controls odors
Removes sulfides
Nonfoaming
Reduction of residuals in whitewater system
Safe decomposition products
No disposal problems
Easy to use
Limited investment cost
Cost-effective system
Elimination of the need to clean the machine over a prolonged break

Based on www.solvaychemicals.com.


Figure 8.9

Structure of ozone molecule.

Table 8.12: Physical properties of ozone

Ozone is the strongest oxidant; has to be generated onsite because of short half-life
Solubility depends on temperature and ozone concentration in the gas phase
Reacts without residuals resulting in oxygen
Ozone works without formation of undesired byproducts
No formation of trihalomethanes
No formation of adsorbable organic halides

radicals. The reactivity of ozone is due to strong oxidizing power of these free radicals (Kim et al., 1999). The main advantage of ozone use consists of its superiority compared to chlorine for the main reasons that it has been reported to be 1.5 times stronger than chlorine and it is acting 3000 times faster than chlorine without producing harmful decomposition products. In 1995, ozone was declared as generally recognized as safe by the Food and Drug Administration for the treatment of bottled drinking water. Moreover, its generally recognized as safe

status was extended to food processing by experts some years later (Voidarou et al., 2007). Because of its high reactivity, it is required only in low concentrations. Similarly to all oxidizing biocides, ozone has some disadvantages like instability and high corrosion potential. Some factors such as temperature and pH affect solubility, stability, and reactivity of ozone. The pH significantly affects the stability of ozone in aqueous solutions. Stability of ozone in solution is the greatest at pH 5.0 and decreases as pH is increased (Kim et al., 1999). In the paper industry, ozone is mainly used for pulp bleaching, water disinfection, and as a final treatment of effluents. Korhonen and Tuhkanen (2000) found ozone to be very effective for controlling bacteria in recycled whitewater. Ozone was studied as a biocide to control microbial growth in a printing paper machine whitewater system from Stora Enso newsprint Varkaus Mills in Finland. Two samples (a cloudy discharge from a disc-filter save-all and a clear filtrate from the same save-all) were treated by ozone dose; about 80% of the aerobic heterotrophic bacteria in the disc save-all cloudy discharge and 90% removal of the aerobic heterotrophic bacteria in the clear filtrate were destroyed (Voidarou et al., 2007). The use of ultrasound with ozone is found to be of great interest because the use of ultrasound in conjunction with biocides offers a greener alternative. Ultrasound is presently employed in several industries such as surface cleaning, medical scanning ultrasonic therapy, food and beverage technology, materials science nanosynthesis (nanotechnology), mineral processing, industrial welding, nondestructive testing, and environmental. Though the energies required for ultrasonic disinfection alone are high there is now commercial equipment available using lower powers that is often combined with ozone (Eadaoin and Timothy, 2008). The ozonation costs are US\$ 1–2/kg O₃ produced, depending on if the ozone equipment already exists. Then, a 99% destruction of the aerobic heterotrophic bacteria will cost from US\$ 0.04–0.15 per m³ whitewater treated. Ozone also functions as a micro flocculating agent to “polish” the water and improve clarity.

Ozone-treated water will maintain better heat transfer efficiencies through reduced biological fouling and increased water clarity. Ozone cannot be stored or transported like other industrial gases because it rapidly decays into diatomic oxygen and should be produced onsite. Because ozone is a short-lived gas molecule that is formed when oxygen reacts with other oxygen molecules to form three parts oxygen (O₃). This reaction requires energy. Ozone generators form ozone by passing dry, clean air through a high-voltage electric discharge (i.e., corona discharge), creating ozone at a concentration of approximately 1%. The corona discharge method is the most common type of ozone generation; these units usually work by means of a corona discharge tube. They are typically cost-effective and do not require an oxygen source other than the ambient air to produce ozone. Temperature and humidity plays a large role in how much ozone is being produced. The important parameter affecting ozone generation efficiency is the gas temperature, which is controlled by cooling water temperature and/or gas velocity. The ozone synthesis is better when the water is cooler. The lower the gas velocity, the higher the concentration, but the lower the net ozone produced. In typical industrial conditions, almost 90% of the effective power is released as heat and needs to be removed by a sufficient cooling water flow.

Because ozone is generated at and injected directly into the water stream, there are no containers of hazardous biocide that can leak, spill, or otherwise cause danger to employee safety. The oxidation power of ozone is actually greater than chlorine (bleach). Although ozone does not remove all minerals or particles, it is extremely effective at containing and eliminating costly microbiological growth, killing bacteria on contact 3100 times faster than chlorine. Ozone's short reaction time also makes it environmentally friendly. Very little residual is maintained within the system because the short-lived reaction takes place immediately after injection into the water stream. This allows for a cleaner and more environmentally friendly discharge to the environment and also an easier path to compliance with discharge permitting.

8.3.9 Glutaraldehyde (1,5-Pentanedial)

Glutaraldehyde is a broad-spectrum biocide and is found to be effective against bacteria, fungi, yeasts, molds, algae, and protozoa. It is extensively used as an antimicrobial agent in a variety of applications such as in cooling water systems, paper-pulp industry, oil field operation, leather tanning industry, poultry industry, cosmetic field, microbiological field, food industry, and the medical area. The extensive use of this biocide is due to being noncorrosive to stainless steel, soft metals, rubber, and glass (Banner, 1995; Herbert, 1995; Lutey, 1995; Walsh et al., 1999). Glutaraldehyde is useful in preventing the formation of slime in Lutey, 1980 all the areas of the papermaking process (Purvis and Tomlin, 1991). Glutaraldehyde is an amber-colored liquid usually supplied in solutions of acidic pH. As with other aldehydes, the two aldehyde groups react readily under suitable conditions, particularly with proteins. It is miscible with water and having melting and boiling points -14°C and 187°C , respectively. It is found to be readily biodegradable and is effective against the aerobic and anaerobic microorganisms including sulfate-reducing bacteria. It is found to be fully compatible with commonly used wet-end additives and significantly reduces the level of microorganisms in both acidic and alkaline systems. It shows more than 90% reduction at 25 ppm and essentially complete kill at 50 ppm in ASTM (American Society for Microbiology) paper slimicide test. It considerably reduces the amount of sulfate-reducing bacteria present in the solutions at any time point Figure 8.10 shows structure of glutaraldehyde. It achieves its biocidal activity by cross-linking the outer proteinaceous layers of the cell in such a way that cellular permeability is changed. The bacterial cell is unable to undertake most, if not all, of its essential functions. The ability of the outer covering of the cell to transport nutrients to the cell and to



Figure 8.10
Structure of 1,5-pentanedial (glutaraldehyde).

remove waste products from the cell is hampered and cell death results (Russell and Chopra, 1996; Simons et al., 2000). The cell walls of all living organisms contain free amine groups (lysine and arginine) that serve as the reactive site for glutaraldehyde attack. Complex cross-links are formed on the cell surface, and as essential cellular functions are disrupted, the cell dies. With increasing pH, more reactive sites for glutaraldehyde attack are formed, and the reaction accelerates. Although glutaraldehyde kills most quickly at alkaline pH levels, it is still effective under acidic conditions. It is also effective against anaerobic bacteria, notably sulfate-reducing bacteria, because it is not inactivated by sulfide.

Glutaraldehyde was first synthesized by Harries and Tank in 1908 (Gorman and Scott, 1980). As with other aldehydes, the two aldehyde groups react readily under suitable conditions, particularly with proteins (Richards and Knowles, 1968). The ratio of monomer to polymer and type of polymer present has been the subjects of numerous publications. The dialdehyde existed as a monomer (25%) in equilibrium with the cyclic hemiacetal. It has been reported that the presence of free aldehyde groups is essential for biocidal activity. From an H-NMR study on commercial (aqueous) acid glutaraldehyde, it has been suggested that the protein cross-linking reactions are possible because of α , β -unsaturated aldehydes (Richards and Knowles, 1968). The pure acid glutaraldehyde underwent very rapid hydration on dissolution in water to give three hydrates in equilibrium. An acetal-like polymer similar to that suggested by Aso and Aito was also shown to exist in acid solution (Aso and Aito, 1962). A scheme depicting glutaraldehyde polymerization in acid and alkaline solutions has been suggested by Gorman and Scott (1980). An increase in temperature produces more free aldehyde in acid solution, whereas in alkaline solution loss of reactive aldehyde groups is possible. Progression to the higher polymeric form could occur with increased time and pH because it has been shown that there is an extensive loss of aldehyde groups from polymerization in alkaline solution (Bowes and Cater, 1966). Therefore loss of reactive aldehyde groups could be responsible for the rapid loss of biocidal activity of alkaline solutions in storage. Increased biocidal activity in heated acid solutions can also be explained by displacement of equilibrium toward the monomer. Glutaraldehyde is an agent that acts as a protein cross-linker and is used as a biocide. It is able to bridge amino acids or H-bonds, thereby modifying the folding of the proteins and stopping its activity (Gorman and Scott, 1980). It is thus likely to react and be consumed by wet-end additives that carry an amine function (Wolf and Sterner, 1972). The dialdehyde reacted with 30–50% of the E-NH₂ groups in the isolated peptidoglycan and it was proposed that two tripeptide side chains could be joined when free amino groups are available (Hughes and Thurman, 1970). Cell wall peptidoglycan (murein, glycopeptide, mucopeptide) contains many chemical groupings capable of reaction with glutaraldehyde. The effect of lysozyme on the isolated wall peptidoglycan of *Bacillus subtilis*, was examined and it was found that although splitting of the lysozyme-sensitive bond occurred, glutaraldehyde-treated peptidoglycan was less sensitive than the untreated polymer to lysis by lysozyme (Hughes and Thurman, 1970). The effect of glutaraldehyde on different

microorganisms is presented in Table 8.13. The stability of glutaraldehyde is affected basically by pH and temperature as shown:

- Glutaraldehyde can be used very effectively up to pH 10. At very high pH (>10.5) glutaraldehyde is still effective, but a shortened half-life may necessitate more frequent dosing.
- Glutaraldehyde is efficacious through a broad temperature range; at higher temperatures glutaraldehyde works faster, although its half-life can be shortened.
- The optimum pH for glutaraldehyde, in terms of rate of efficacy and half-life, is in the range of 7–9, which encompasses most use conditions.
- Adverse storage conditions may impact product quality in a nonhazardous way.

Glutaraldehyde is supplied as follows:

- As an aqueous solution in concentrations ranging from 14% to 50% actives.
- As a blend with quaternary amines combining two unique biocides with two different modes of action to deliver synergy and even greater efficacy.
- As freeze-protected blends that offer high performance in extreme conditions.

Aqueous solutions of glutaraldehyde do not contain or require stabilizers, salts, or heavy metals, which may concentrate in closed systems. Table 8.14 shows the physical properties of 50% aqueous glutaraldehyde.

Table 8.13: Effect of glutaraldehyde on different microorganisms

Microorganism	Time for complete kill (hours)	ppm
<i>Escherichia coli</i>	1.0	50
<i>Enterobacter aerogenes</i>	1.5	45
<i>Pseudomonas aeruginosa</i>	1.0	25
<i>Klebsiella pneumoniae</i>	1.0	50
<i>Staphylococcus aureus</i>	1.0	50
<i>Candida albicans</i>	7.0	100

Control cfu for all organisms 10^5 ; pH = 7.0.

Based on www.prirodni-akvarium.cz/clanky/Glutaraldehyde.pdf.

Table 8.14: Physical properties of 50% aqueous glutaraldehyde

Specific gravity	1.129 ($H_2O = 1$)
Boiling point	100.5 °C
Freezing point	–21 °C
Vapor pressure at 20 °C	0.20 mm Hg (active ingredient)
Solubility in water	100%
Flash point	None
Exposure limits	0.1 ppmv ceiling (Union Carbide) 0.05 ppmv ceiling (ACGIH)

ACGIH, American Conference of Governmental Industrial Hygienists; ppmv, parts per million volume.

Based on www.prirodni-akvarium.cz/clanky/Glutaraldehyde.pdf.

Glutaraldehyde-based formulations are extremely effective in controlling the growth of unwanted organisms. However, the minimal impact of glutaraldehyde on the natural environment is just as important as its biocidal efficacy. Several studies have been conducted to determine the acute toxicity of glutaraldehyde to aquatic organisms, both fresh water and marine/estuarine. In acute toxicity studies of glutaraldehyde in three aquatic species, the no observable effect concentrations ranged from 2.5 to 0.029 mg/L (algae) to 9–24 mg/L (*Daphnia magna*) in freshwater and marine studies, respectively. In chronic studies, the no observable effect concentrations ranged from 0.31 mg/L (algae) to 4.25 mg/L (*Daphnia magna*). This suggests that the environmental toxicity of glutaraldehyde does not increase significantly with repeated exposure. Glutaraldehyde belongs to the aldehydes chemical class whose properties clearly differ. Unlike formaldehyde, all available long-term animal data clearly reveal that glutaraldehyde is not carcinogenic. Several regulatory and advisory agencies have set occupational exposure limits for glutaraldehyde. Users should ensure that any exposure does not exceed the limit applicable. However, a limit as such does not prevent the use of glutaraldehyde-based products in any application. Use as a high-level disinfectant on medical devices has led to cases of eye, nasal, respiratory, and skin irritation and dermal sensitization basically because of poor control of exposure following spills. In some cases, occupational asthma has been reported, although the available data do not suggest that exposure up to the limit value induces such effects. Products based on glutaraldehyde are effective against gram-positive and gram-negative bacteria, fungi, and a variety of viruses (including infectious bursal disease, porcine reproductive and respiratory syndrome virus, hog cholera virus, human corona virus, Newcastle disease virus, avian reovirus, avian rotavirus, and strains of avian influenza virus). This wide spectrum of biocidal activity supports the many diverse applications. [Tables 8.15 and 8.16](#) show features and limitation of glutaraldehyde, respectively.

Protectol GA 50 biocide marketed by BSF is a 50% solution of glutaraldehyde. It is very effective against a broad spectrum of bacteria and fungi common to the papermaking industry and is useful in the prevention of slime buildup in all areas of the manufacturing process. Protectol GA 50 has a rapid speed of kill, is cost-effective, and easy to use. In paper processing, the principal benefit of Protectol GA 50 is that it can be used as a stand-alone product.

Table 8.15: Features of glutaraldehyde

Broad-spectrum efficacy
Quick kill under alkaline conditions
Highly effective against biofilm, sulfate-reducing bacteria and <i>Legionella</i>
Compatible with dispersants, surfactants and most WT chemicals, including CMIT/MIT
Compatible with halogens and other WT additives
Readily biodegradable at concentrations <5 ppm
Does not contain or release formaldehyde
Kills via cross-linking proteins in cell wall

Based on Mirrico seminar, Kazan, September 2011.

Table 8.16: Limitations of glutaraldehyde

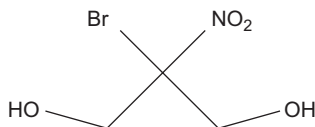
Polymerizes under alkaline and high temperature conditions
Shows weak efficacy versus fungi and algae
Stability with ammonia and alkaline pH
Deactivated by bisulfites
Evaporation potential increases with temperature and/or aeration

Based on Mirrico seminar, Kazan, September 2011.

Table 8.17: Minimum inhibitory concentrations (MIC) of Protectol GA 50 biocide

Test organism	MIC (ppm)
<i>Staphylococcus</i> spp.	50
<i>Bacillus</i> spp.	1250
<i>Desulfovibrio</i> spp.	60
<i>Pseudomonas</i> spp.	150–250
<i>Candida</i> spp.	1250
<i>Aspergillus</i> spp.	475

www2.basf.us/biocides/pdfs/PIB_Brochure.pdf.

**Figure 8.11**

Structure of 2-bromo-2-nitropropane-1,3-diol (Bronopol).

It is extremely effective against slime-forming bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus* spp. found in fouled papermaking systems. The ability of Protectol GA 50 to rapidly reduce the level of microorganisms present in a typical paper making process is excellent. The minimum inhibitory concentrations (MIC) of Protectol GA 50 biocide are presented in [Table 8.17](#).

8.3.10 Bronopol (2-Bromo-2-nitropropane-1,3-diol)

Bronopol (2-bromo-2-nitropropane-1,3-diol) is a white crystalline odorless substance melting at about 130 °C. It is soluble in water, lower alcohols, AA, diethyl ether, and ethyl acetate, but weakly soluble in chloroform and acetone, and practically insoluble in hydrocarbon solvents ([Legin, 1996](#)). [Figure 8.11](#) shows the structure of 2-bromo-2-nitropropane-1,3-diol (Bronopol). Its solubility values in some of the solvents are as follows (wt./vol.% at 22–25 °C):

- Water, 28
- Methyl alcohol, 89
- Ethyl alcohol, 56

- Isopropyl alcohol, 41
- Ethylene glycol, 61
- Methyl carbitol, 54
- 1,2-propylene glycol, 52
- Dipropylene glycol, 48
- Polyethylene glycol, 300
- Diethyl sebacinate, 10
- Isopropyl myristate, mineral oil, and vegetable oils, less than 0.5

Aqueous solutions of the pure compound have a pH of 5.0–5.5. This is explained by the mobility of hydroxyl hydrogen atoms. The compound in the solid state can be stored for 3 years and longer, and it is not affected by factors such as daylight, air humidity (up to relative humidity of 90%), and temperature (up to 45 °C) (Legin, 1996; Bryce et al., 1978). However, aqueous solutions of Bronopol are stable only in the cold, provided that the acidity is sufficiently high. An increase in the pH and temperature leads to decomposition of the compound. This is due to the splitting of formaldehyde. The initial process in the decomposition of Bronopol appears to be a retro-aldol reaction with the liberation of formaldehyde and the formation of bromo nitroethanol (Bryce et al., 1978). Bromonitro ethanol itself is significantly less stable than Bronopol and in the range of conditions investigated its maximal concentration did not exceed 0.5% of the initial Bronopol concentrations. Simultaneously a second-order reaction involving Bronopol and formaldehyde occurs to give 2-hydroxymethyl-2-nitro-1,3-propanediol. The antimicrobial activity of Bronopol is mostly because of the presence of electron deficient bromine atoms in the molecules, which shows oxidation properties rather than the ability to liberate formaldehyde (Legin, 1996). The mechanism of the antimicrobial effect of Bronopol consists of the cross-linking of sulfohydryl groups of dehydrogenase enzymes occurring on the surface of microbial cells. The disulfide bridges block microbial metabolism.

BSF has launched Myacide AS biocide. It is the industrial grade of 2-bromo-2-nitropropane-1,3-diol or Bronopol. It provides highly effective antimicrobial activity for use in diverse and demanding industrial biocide applications, combining well-proven efficacy with important environmental safety characteristics. The main benefits of Myacide AS in the paper industry are its established performance as a slimicide active ingredient in mill process water and as an effective preservative for mill additives. The ideal physical and microbiological properties of Bronopol place it in a new generation of products, which can be seen replacing older chemistries. The product contains a minimum of 98% 2-bromo-2-nitropropane-1,3-diol. Bronopol is highly effective, particularly against aerobic slime-forming bacteria such as *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., and the *Enterobacter* spp. Table 8.18 compares the MIC of Bronopol against six key microorganisms. Figure 8.12 shows the efficacy of Bronopol in preserving a typical paper mill additive based on calcium carbonate. The procedure used was ASTM E 723-91 at pH 7.5 with a mixed inoculum of *P. aeruginosa*, a second (1,2-benzisothiazolin-3-one-resistant) *Pseudomonas* species, *Enterobacter cloacae*, and *Klebsiella aerogenes*. Myacide AS at a level of >20 ppm was able to control this mixed inoculum for 6 weeks following a single initial challenge

Table 8.18: Minimum inhibitory concentrations (MIC) of Bronopol

Test organism MIC (ppm)	MIC (ppm)
<i>Staphylococcus</i> spp.	12.5–50
<i>Bacillus</i> spp.	12.5–50
<i>Desulfovibrio</i> spp.	12.5–50
<i>Pseudomonas</i> spp.	12.5–50
<i>Candida</i> spp.	400
<i>Aspergillus</i> spp.	3200

www2.basf.us/biocides/pdfs/PIB_Brochure.pdf

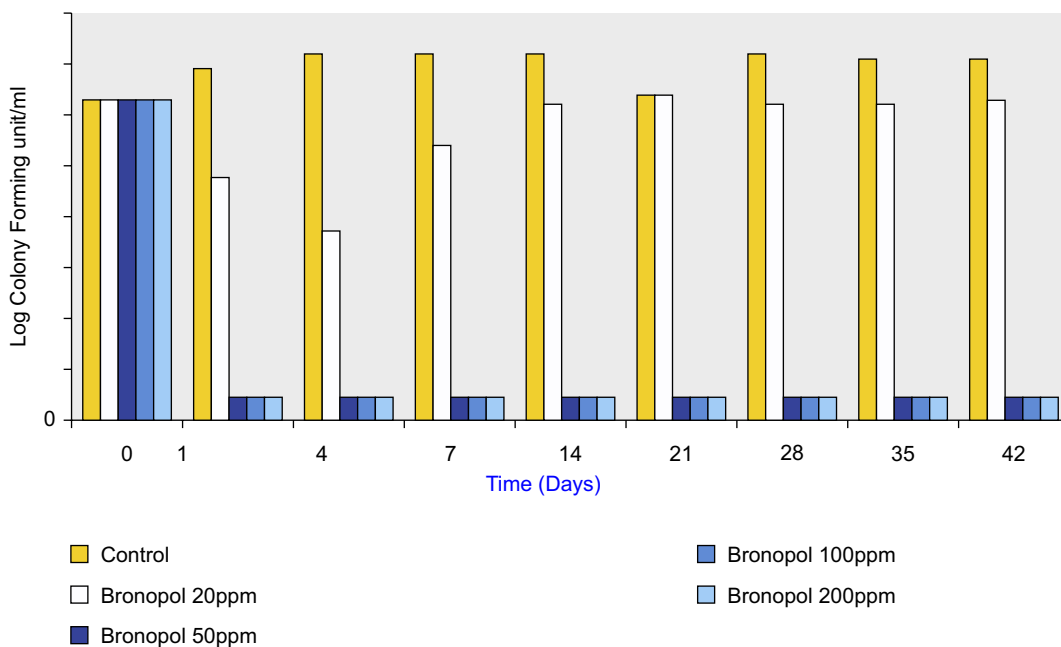


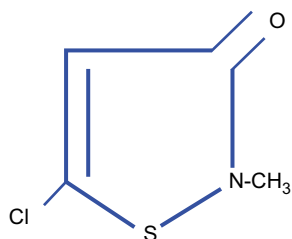
Figure 8.12

Efficacy of Bronopol in preserving a typical paper mill additive based on calcium carbonate. Based on Specialty Chemicals by BASF, Paper industry biocides www2.basf.us/biocides/pdfs/PIB_Brochure.pdf.

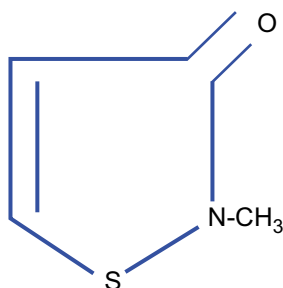
of 106 cfu/mL and a reinoculation at 21 days. 1,2-benzisothiazolin-3-one at levels of up to 200 ppm active was unable to control the *Pseudomonas* growth.

8.3.11 5-Chloro-2-Methyl-4-Isothiazolin-3-One/2-Methyl-4-Isothiazolin-3-One

5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-methyl-4-isothiazolin-3-one (MIT) are active ingredients of Kathon WT biocides. These biocides are marketed by Dow Chemical Company. Figures 8.13 and 8.14 show the structures of CMIT and MIT. It is a high-performance paper mill slimeicide with a broad spectrum of activity that can cope with the rapid changes in microbial flora that occur in different papermaking systems. It penetrates and kills

**Figure 8.13**

Structure of 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT).

**Figure 8.14**

Structure of 2-methyl-4-isothiazolin-3-one (MIT).

microorganisms in the biofilm and is not inactivated by the high level of suspended organic solids found in paper mill water. It also provides cost-effective microbial control. Kathon WT has amber-gold color, is completely soluble in water, and has a mild odor and specific gravity of 1.32 at 20°C. Kathon is stable over a wide range of conditions found in cooling water and paper mill applications. It is an extremely effective, broad-spectrum microbiocide that causes an immediate inhibition of growth on coming in contact with a microorganism. It is effective over a wide pH range and is therefore ideal for use in the alkaline conditions that exist in multicycle cooling towers and modern papermaking (Divkovic et al., 2005). It is found to be compatible with chlorine, corrosion, and scale inhibitors and most anionic, cationic, and nonionic formulations at normal-use levels. It causes immediate inhibition of growth on coming in contact with a microorganism. The growth inhibition rapidly becomes irreversible and results in cell death. Even before death occurs, the Kathon-treated organism is unable to synthesize degradative enzymes or the exopolymers that facilitate adhesion and biofilm formation. Growth inhibition rapidly becomes irreversible and results in cell death when essential proteins are progressively oxidized. It controls the wide variety of algae, bacteria, and fungi found in industrial water systems. Such a broad-spectrum product reduces inventory and handling costs, lowers operator training expenses, and reduces the risk of dosing error. Effective control of such a wide variety of microorganisms at levels as low as 1 ppm active ingredient provides an unrivalled and cost-effective treatment. It readily penetrates the surface of adhering biofilm to give effective control of sessile microorganisms. When diluted below use concentrations, it is readily

Table 8.19: Features and benefits of CMIT/MIT

<i>Fast-acting</i>
Provides immediate control
<i>Broad-spectrum activity</i>
Effective against bacteria, algae, and fungi. Effective versus <i>Legionella</i> , biofilm, sulfate-reducing bacteria
<i>Stable over a wide range of pH and temperature</i>
Effective under conditions typically encountered in most processes
<i>Clear, water soluble liquid</i>
Fully water soluble at use levels and easy to dose
<i>Broad chemical compatibility</i>
Compatible with most cooling water and paper mill additives and biocides
<i>Low use rates</i>
Cost-effective
<i>Biodegradable and does not produce adsorbable organic halides or formaldehyde</i>
Environmentally friendly

Based on Mirrico seminar, Kazan, September 2011.

Table 8.20: Limitation of CMIT/MIT

Poor stability above pH 9 and >40 °C
Poor stability with nucleophiles and reducing agents
Poor stability above pH 9 and at temperature higher than 40 °C
Perceived weakness versus sulfate-reducing bacteria
Slow killing
Safe handling concerns/sensitization/burns
New solid version will address safety issues

Based on Mirrico seminar, Kazan, September 2011.

biodegradable. Their decomposition does not lead to the presence of chlorinated organics in the environment. [Tables 8.19 and 8.20](#) show features and benefits of CMIT/MIT.

[Figure 8.15](#) presents a case history of biocide treatment in a newsprint mill where biocide addition was at the broke towers. Using carbamate, bacterial counts in the broke pulp were unacceptably high. After changing to a cost equivalent level of Kathon WT, bacterial counts in the broke were significantly reduced and downtime from contamination was minimized.

8.3.12 2,2-Dibromo-3-Nitrilopropionamide

DBNPA is a powerful biocide with two exceptional properties: it kills microorganisms immediately upon addition and it degrades rapidly ([Exner et al., 1973](#)). It is a white crystalline powder having melting point of 124.5 °C, water solubility 15,000 mg/L at 20 °C, and vapor pressure 9.00×10^{-4} mm Hg at 20 °C ([Norstrom et al., 2009](#)). [Figure 8.16](#) shows the structure of DBNPA. It is intended for commercial use in pulp, paper, and paperboard mills; industrial cooling water systems; industrial air-washer systems; enhanced oil and gas

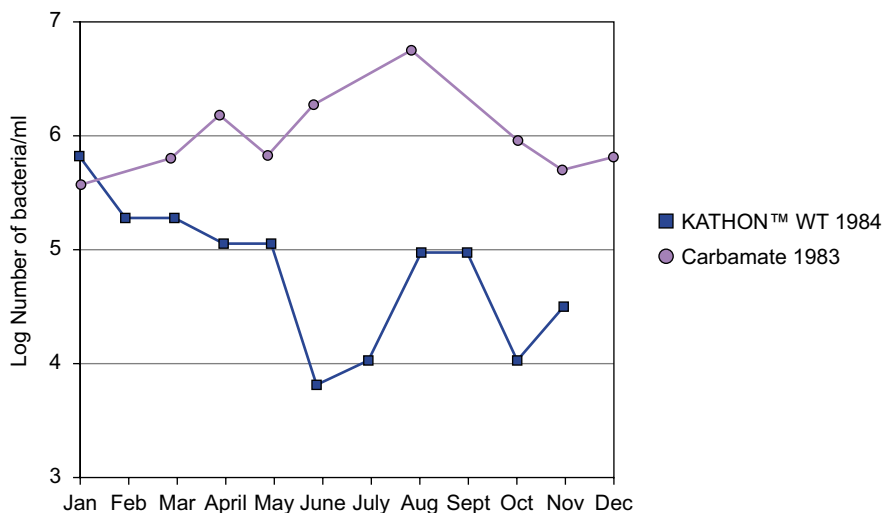


Figure 8.15

Comparative efficacy of KATHON WT and carbamate in a paper mill producing newsprint. *From KATHON™ WT water treatment microbicide. Reproduced with permission from the Dow Chemical Company.*

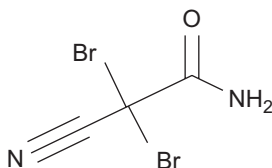


Figure 8.16

Structure of 2,2-dibromo-3-nitrilopropionamide (DBNPA).

recovery systems; metal-working fluid systems; and the paint and coatings industries. It can also be added to finished products as a preservative enhancer. It controls algae, bacteria, and fungal growth. DBNPA has low vapor pressure and high water solubility that makes the compound be retained mainly in the water phase. But, DBNPA has a short half-life and is rapidly degraded in water by hydrolysis. DBNPA is not an oxidizing biocide and is bromine releasing. DBNPA does act similarly to the typical halogen biocides. The liquid formulated DBNPA is an oxidizer, and the solid formulated DBNPA as a tablet is not an oxidizer. The liquid formulation is oxidizing because of the HOBr in the formulation. DBNPA readily degrades under alkaline conditions. It is sensitive to ultraviolet light and nucleophilic substances. It is uncharged and non-surface-active and it seems unlikely to interact with wet-end additives (Huber et al., 2010). DBNPA dissolves in water, forming a relatively stable solution in an acid pH range. Its unusual solubility and stability in polyethylene glycol (average molecular weight, 200 Da) make this glycol a preferred solvent. Aqueous solutions hydrolyze under alkaline conditions with the rate of decomposition increasing with the alkalinity.

However, the rate of hydrolysis is not fast enough to interfere with the antimicrobial activity of fresh, alkaline (pH 7–9.5) solutions (Wolf and Sterner, 1972).

Although DBNPA is compatible with many chemical classes, including oxidizing agents, it will react readily with nucleophilic agents and sulfur-containing reducing agents. The facile reaction of DBNPA with sulfur-containing nucleophiles common to microorganisms, such as glutathione or cysteine, is the basis of its mode of antimicrobial action. DBNPA is therefore not a typical oxidizing or halogen-releasing biocide. Unlike other thiol-reactive biocides, its action is such that thiol-based amino acids, like cysteine, are oxidized beyond the formation of disulfide species. This reaction irreversibly disrupts the function of cell-surface components, interrupting transport across cell membranes and inhibiting key biological functions. DBNPA degrades rapidly by both nucleophilic and hydrolytic pathways to relatively nontoxic products. The rate of hydrolysis of DBNPA is strongly pH-dependent: at pH 6.0 and 25 °C, the DBNPA molecule has a half-life of 155 h (about 6.5 days), but at pH 8.0 and 25 °C, its half-life is about 2 h. The ultimate degradation products of DBNPA are ammonia, carbon dioxide, and bromide ion. The mechanism for the environmental degradation of a DBNPA has been described by Exner et al. (1973). There are two competing pathways:

1. DBNPA ~ DBAM (dibromoacetamide)
2. DBNPA ~ CAM (cyanoacetamide).

The second pathway was defined as occurring in the presence of nucleophiles or sunlight. Table 8.21 and 8.22 show features and limitations of DBNPA. It can be considered rapidly degradable, would be removed by wastewater treatment facilities, and would not persist in the environment. It does not accumulate in the food chain. However, it is highly toxic (US classification)/very toxic (EU classification) to aquatic organisms on an acute basis.

8.3.13 2-n-Octyl-4-Isothiazolin-3-One or *Kathon 893*

OIT (2-n-octyl-4-isothiazolin-3-one) is marketed by Dow Chemical Company. It is a yellow liquid miscible in water and oil and stable in light and pH 9.5. OIT exhibits excellent fungistatic and fungicidal activity against fungi, including yeasts, mold, and gram-positive bacteria, and limited activity against gram-negative bacteria. OIT also belongs to the isothiazolinone group which in general all are electrophilic molecules containing an activated N-S bond that enables them to react with nucleophilic cell entities thus exerting an antimicrobial action (Alaxender, 2002). Figure 8.17 shows the structure of OIT. This biocide makes use of a two-step mechanism that involves rapid growth inhibition leading to a loss of cell viability. Growth inhibition is the result of rapid disruption of the central metabolic pathways of the cell by inhibition of several specific enzymes, including dehydrogenases. The essential enzymes that are affected are associated with the nutrient metabolism, Krebs cycle, and energy generation. The main physiological activities that are rapidly inhibited in microbial cells are respiration (oxygen consumption), energy generation (ATP synthesis), and growth (assimilation). Most of these major

Table 8.21: Features of DBNPA

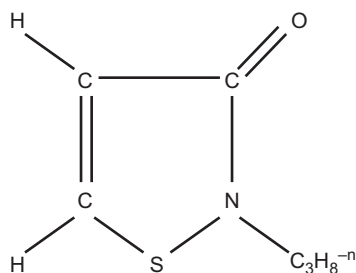
Shows broad-spectrum efficacy
Extremely fast-acting
Effective at lower dose levels
Highly effective against biofilm and <i>Legionella</i>
Easy to dose liquid
Noncorrosive at in-use concentration
Low environmental impact
Short half-life at highly alkaline pH
Kills via disruption of respiration and metabolism; reactions with sulfhydryls

Based on Mirrico seminar, Kazan, September 2011.

Table 8.22: Limitation of DBNPA

Limited shelf life (6 months)
Weak versus algae and fungi
Not compatible with strong nucleophiles and reducing agents
Low solubility in water
Not ultraviolet stable
Occasionally referred to as an oxidizer

Based on Mirrico seminar, Kazan, September 2011.

**Figure 8.17**

Structure of 2-n-octyl-4-isothiazolin-3-one (OIT).

enzymes are present in both aerobic and anaerobic microorganisms, which shows the broad spectrum nature of this biocide. Inhibition of cellular activity and growth is rapid within minutes. However, cell death (cidal activity) is observed after several hours of contact. Generally, the higher the concentration of biocide, the shorter the contact time needed for more complete kill. Cell death results from the progressive loss of protein thiols in the cell from one of the multiple pathways. As cell metabolism is disturbed, free radicals are produced, which also results in cell death. This exceptional mechanism results in its broad spectrum activity.

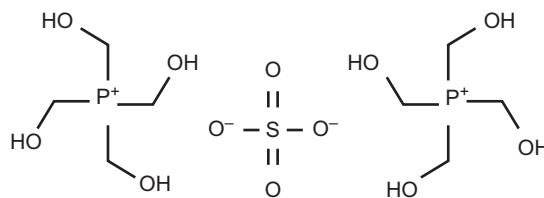


Figure 8.18

Structure of tetrakis (hydroxymethyl) phosphonium sulfate (THPS).

8.3.14 Tetrakis (Hydroxymethyl) Phosphonium Sulfate

The USEPA registered a new class of antimicrobial chemistry for use in papermaking. This is based on the biocidal molecule tetrakis hydroxymethyl phosphonium sulfate (THPS) (Hack et al., 1997). THPS biocides are classified by Department of Transportation as nonhazardous because they have very low acute toxicity in the environment. THPS is fully water soluble and a clear colorless liquid. It is found to be highly effective against sulfate-reducing bacteria having an odor that resembles aldehyde and is stable for 14 days at temperatures 21–54 °C (WHO, 2002). THPS is a quaternary phosphonium salt having the structure shown in Figure 8.18 (Haack and Downward, 1997). Aqueous solutions of THPS are acidic (pH 3.2) because of the small dissociation of THPS to tris(hydroxymethyl)phosphine, $P(CH_2OH)_3$, formaldehyde, and sulfuric acid (Thomas et al., 2007). THPS is also readily biodegradable and has no potential to bioaccumulate. Another environmental benefit is that THPS is rapidly oxidized in the environment to trishydroxymethylphosphine oxide (THPO), which has a very low aquatic toxicity and is not considered to present an environmental hazard (Haack and Downward, 1997). The following data show the low toxicity of THPO.

- Rainbow trout, 96 h LC50: >5000 mg/L
- Daphnia magna, 48 h EC50: >1000 mg/L
- Skeletonema costatum EC50: 2090 mg/L

Both THPS and THPO will also photodegrade in the environment. Based on these and other data, compared with conventional biocides, THPS offers genuine environmental benefits. Table 8.23 shows effect of THPS against *Enterobacter aerogenes* and sulfate-reducing bacteria.

THPS degrades rapidly on discharge to a molecule that is virtually nontoxic, thus reducing the risk of pollution and/or harm to biological effluent treatment plants. These biocides are fast-acting and are effective against sulfate-reducing bacteria and biofilms. The most exceptional property of THPS is its ability to combine broad spectrum antimicrobial effectiveness with a relatively benign human and environmental toxicity profile. The nonfoaming THPS molecule can be monitored on-site with a simple and rapid titration procedure to ensure proper dosing levels. Laboratory tests showed that up to 200 ppm of THPS formation could safely be dosed to the paper mill without adversely affecting the effluent treatment plant

Table 8.23: Effect of THPS against *Enterobacter aerogenes* and sulfate-reducing bacteria

<i>Enterobacter aerogenes</i>			
THPS concentration (ppm a.i.)	Surviving bacteria per milliliter after stated exposure time ^a		
	2 h	6 h	24 h
0 (control)	2.3×10^5	1.3×10^5	1.9×10^6
15	4.0×10^4	1.0×10^0	0
37.5	1.0×10^0	1.0×10^0	0
75	0	0	0
150	0	0	0

<i>Sulfate reducing bacteria</i>		
THPS concentration (ppm a.i.)	Surviving sulfate reducing bacteria per milliliter after stated exposure time ^b	
	6 h	24 h
0 (control)	8.8×10^6	1.4×10^7
10	1.1×10^5	1.4×10^7
25	2.0×10^0	0
50	0	0
100	0	0

^aThe initial bacterial level was 1.8×10^6 .^bThe initial SRB level was 2.0×10^6 .

Based on Haack et al. (1997).

Table 8.24: Effect of THPS on activated sludge in the biological effluent treatment plant

THPS dose (ppm)	Oxygen demand (%O ₂ /min)
0 (control)	40
100	44
200	39
300	20

Based on Haack et al. (1997).

(Haack et al., 1997) (Table 8.24). This indication was confirmed during the plant trial, which demonstrated that microbial control could be achieved in the process at a dose rate of only 9.6 ppm THPS. Hydrogen sulfide levels were controlled at acceptable levels. There were no detrimental effects in the effluent treatment plant. Table 8.25 shows features and benefits of THPS and Table 8.26 shows limitation of THPS.

8.3.15 3.8 Dazomet (3,5-Dimethyltetrahydro-1,3,5-Thiadiazine-2-Thione)

Dazomet, a heterocyclic compound, is used as a slimicide in paper mills; a material preservative treatment for coatings, adhesives, epoxy flooring compounds, slurries, and high viscous suspensions; a biocide treatment used during petroleum operations; a biocide treatment to

Table 8.25: Features and benefits of THPS

<i>Active against sulfate-reducing bacteria, algae, and Legionella</i>
Useful for a wide range of industrial applications
<i>Broad-spectrum and fast-acting biocide</i>
Control of wide range of microorganisms
<i>Dissolves iron sulfide</i>
Reduces iron sulfide related problems, fouling of equipment
<i>Low dosages</i>
Cost-effective
<i>Favorable aquatic toxicity</i>
Very low impact on ecology and minimal effect on environment
<i>No organic solvents</i>
Safety in use; completely water miscible
<i>Nonfoaming</i>
Easy to use in high-flow system

Based on Mirrico seminar, Kazan, September 2011.

Table 8.26: Limitation of THPS

Not compatible with oxidizing biocides
Cationic properties react with anionic inhibitors
Releases formaldehyde rapidly
Unstable at high pH
Issues with use of THPS in high calcium waters

Based on Mirrico seminar, Kazan, September 2011.

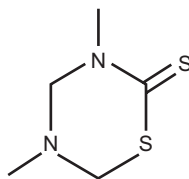


Figure 8.19
Structure of Dazomet.

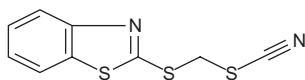
recirculating cooling water systems; and a remedial wood treatment to utility poles. Dazomet is considered moderately toxic on an acute oral basis to both birds (lethal dose 50% [LD50]=424 mg/kg) and mammals (LD50=415 mg/kg). It has a melting point of 104–105 °C, flash point 156 °C, water solubility <0.1 g/100 mL at 18 °C, and storage temperature 0–6 °C. Its structure is shown in [Figure 8.19](#).

Dazomet is degraded by hydrolysis in an aqueous solution within a period of days ([Chervenak et al., 2005](#)). Dazomet, a biocide with good activity in principle, suffers in particular in products adjusted to an alkaline pH (e.g., talcum, calcium carbonate slurries) rapid

Table 8.27: Minimum inhibitory concentrations (MIC) of Dazomet biocide

Test organisms	MIC (ppm)	
	20 °C	40 °C
<i>Staphylococcus aureus</i>	500	3.9
<i>Escherichia coli</i>	500	62.5
<i>Proteus mirabilis</i>	250	15.6
<i>Pseudomonas aeruginosa</i>	250	31.3
<i>Candida albicans</i>	500	7.8

www2.basf.us/biocides/pdfs/PIB_Brochure.pdf.

**Figure 8.20**

Structure of TCMTB (2-(thiocyanomethylthio)benzothiazole).

degradation with release of toxic and highly odorous gases (Sismanoglu et al., 2004). Dazomet is rapidly degraded in aqueous media to carbon disulfide, formaldehyde, and methylamine, with half-lives in the order of 3–20 days. However, in moist soil it degrades mainly to methyl isothiocyanate. It is unstable and decomposes to methylamine in water, probably via thiocarbamic acid. Multiple products are observed including methyl isocyanide, sulfur dioxide, hydrogen sulfide, N-methyl formamide methylamine, and carbonyl sulfide. Methyl isocyanide in turn degrades to methyl isocyanate (Ruzo, 1982).

Protectol DZ biocide marketed by BSF is the trade name for Dazomet. Within the pulp and paper industry, it is used both as a preservative for mill additives and as an active slimicide to control growth in the process water. It boasts excellent antimicrobial activity. Protectol DZ is particularly active against slime-forming organisms and sulfate-reducing bacteria, which can be responsible for corrosion. The most important benefits of Dazomet in the pulp and paper industry are its rapid speed of kill and broad-spectrum activity. Dazomet is extremely effective against slime-forming and spoilage organisms such as *Pseudomonas* spp., *Klebsiella* spp., *S. aureus*, *Aspergillus niger*, and *Candida albicans*. The MIC of Dazomet is displayed in Table 8.27.

8.3.16 2-(Thiocyanomethylthio)Benzothiazole

TCMTB (2-(thiocyanomethylthio)benzothiazole) is used as a slimicide and paper coating preservative for controlling bacteria, fungi, and yeasts that cause deterioration of paper and paperboard products and used to preserve paper-adhesive formulations. In its pure form, TCMTB is a white crystalline, whereas technical grade TCMTB is a black viscous liquid. Figure 8.20 shows the structure of TCMTB. It forms the active ingredient of the commercial fungicide Woodstate 30WBR. It has low aqueous solubility having boiling point: >120 °C,

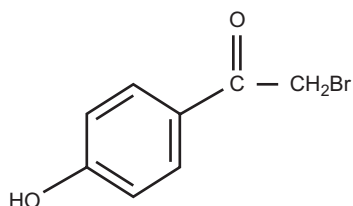
melting point: $<-10^{\circ}\text{C}$, relative density (water = 1): 1.4, solubility in water, 100 g/mL: 0.0033 (Cserjesi and Johnson, 1982). The substance decomposes on heating in the presence of sulfide or when exposed to sunlight producing toxic fumes including hydrogen cyanide, nitrogen oxides, and sulfur oxides (Meneses et al., 2005). Hydrolysis and/or photolysis of TCMTB results in 2-MBT, which can photolyze to benzothiazole and 2-hydroxybenzothiazole or undergo biomethylation to 2-(methylthio)benzothiazole. TCMTB has the following properties.

- Stable at pH 5.0
- Slowly hydrolyzed at pH 7.0
- Rapidly hydrolyzed at pH 9.0

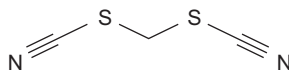
The major breakdown pathway of TCMTB results from photolysis (Nawrocki et al., 2005). TCMTB products are used in commercial/institutional premises and residential and public access areas. As an antimicrobial pesticide, TCMTB is used largely as a materials preservative (e.g., leather products and hides, pulp/paper products, latex, wallpaper, paints, carpets). TCMTB is a slimicide regulated by the Food and Drug Administration (21 CFR 176.300) for controlling bacteria, fungi, and yeasts, which cause deterioration of paper and paperboard products. TCMTB is also used as a fungicide for commercial and on-farm seed treatment. It is used as pulp/paper slimicide, paper-adhesive preservative, and in paper coating. It belongs to chemical family thiazole and its common name is TCMTB or TCMB; its empirical formula is $\text{C}_9\text{H}_6\text{N}_2\text{S}_3$; and another of its names is 2-(benzothiazolythio)methyl thiocyanate.

8.3.17 2-Bromo-4-Hydroxyacetophenone

BHAP (2-bromo-4-hydroxyacetophenone) is an organobromine compound and is a good biocide for bacterial slimes. Its common name is 2-bromo-4'-hydroxyacetophenone and its chemical name is ethanone 2-bromo-1-(4-hydroxyphenyl). There are three products—Busan 90, 93, and 1130—containing the active ingredient bromohydroxyacetophenone. Buckman Laboratories, Inc., manufactures these three products. They are used in the control of slime-forming bacteria, deterioration/spoilage bacteria, fungi, and slime-forming fungi in paper mills and water systems. They have also been used to inhibit the growth of bacteria and fungi that cause the microbiological degradation of papermaking chemicals. It is a reddish brown, viscous, odorless liquid, having a boiling point of $139.1 \pm 0.7^{\circ}\text{C}$ at 737.9 mm Hg and solubility 0.248 ± 0.07 g/100 mL water. It is found to be stable under all storage conditions. At a pH 5.0, 7.0, and 9.0, BHAP has a hydrolytic half-life of 272, 250, and 173 h, respectively. Figure 8.21 shows the structure of BHAP. It is useful for situations requiring continuous or semicontinuous dosing at low levels, such as once-through cooling systems, where the dose rate is only 1–3 mg/L. It is supplied as a 30% active biocide. The dose rate for recirculating cooling systems is typically 10–20 mg/L, but could possibly reach up to 80 mg/L in fouled systems. Considerably higher dose rates are required for algal and fungal slimes. Because BHAP is not pH-dependent, it is effective at high pH levels. However, BHAP has a relatively long half-life, typically 175–250 h, which may affect its potential for cooling system bleed discharge. Other organobromine group products with similar biocidal mechanisms include:

**Figure 8.21**

Structure of 2-bromo-4-hydroxyacetophenone (BHAP).

**Figure 8.22**

Structure of methylene bis(thiocyanate).

bisbromo acetyl butene and β -bromo- β -nitrostyrene. An example of BHAP is BRM10 from Buckman Laboratories, Inc. (Frayne, 2001). BHAP photodegrades in water with a half-life of less than 2 days. Also, BHAP's half-life in aerobic aquatic environments is 2.5 days.

8.3.18 Methylene Bis(Thiocyanate)

MBT is used as a biocide in a number of applications. Its major use is in water-cooling systems and paper mills as an inhibitor of algae, fungi, and bacteria. It is also used for preservation of paints, adhesives, synthetic polymer lattices, and latex emulsions; antimicrobial treatment of water-thinned products, thickeners, and slurries; preservation of wood and wood products; and preservation of leather and leather products and oil well brines and drilling mud. MBT is a yellow to light orange solid that melts at 105–107°C. It has limited solubility in water (<1 mg/mL) but is soluble in organic solvents and therefore it is usually formulated with dispersants or supplied as a water slurry. Its molecular formula is $\text{CH}_2(\text{SCN})_2$ and molecular weight is 130. Figure 8.22 shows the structure of MBT. It maintains long-term effects and is applicable to broad pH value and temperature ranges. MBT is pH-sensitive and rapidly hydrolyzes in the pH range above 8.0. For this reason, it is not recommended for use in systems where the recirculating water pH generally exceeds 8.0 (Mass-Diepeveen and Van Leeuwen, 1988). Not much is known about the mechanism of MBT responsible for biocidal activity. It has been suggested that it inhibits cell respiration. Because it is a competitive microbiocide, it inactivates the electron transfer cytochromes of the microorganisms. The thiocyanate fragment of the methylene ester of the thiocyanic acid reacts to blocking the transfer of electrons in the microorganism, which results in cell death.

8.3.19 Other Biocides

Patent WO 1999,043209 A1 by Buckman Laboratories (King et al., 1999) describes potentiation of biocide activity using a diethanolamide. In the method, at least one biocide and at least one

diethanolamide are applied to a substrate or aqueous system subject to the growth of microorganisms. The diethanolamide is applied in an amount effective to increase the biocidal activity of the biocide. Biocidal compositions are described where the biocide and the diethanolamide are present in a combined amount effective to control the growth of at least one microorganism. The combination of the biocide and the diethanolamide is particularly useful as a biocide in the leather industry, the lumber industry, the papermaking industry, the textile industry, the agricultural industry, and the coating industry as well as in industrial process waters.

Pereira et al. (2001) studied reduction of biofouling in paper production processes by using a carbamate-based biocide as a retention agent. This biocide was used to modify the surface properties of the microbial cells to promote their attachment to the cellulose fibers and to prevent the bacteria from developing biofilms on the equipment. About 75% of the cells were retained by the fibers. The effect of glutaraldehyde, another traditional biocide in pulp mills, was also tested and it was concluded that this biocide did not modify the surface charge of the bacteria. Contrary to the glutaraldehyde, the carbamate-based biocide modifies the surface electrical charge of the microbial cells, making them more positive. Therefore, this biocide may promote cell aggregation when the electrostatic repulsion becomes low or nonexistent (i.e., when the zeta potential is close to zero). This happens for carbamate concentrations of 100 mg/L and 200 mg/L, when the pH is 5.9, and for concentrations of 200 mg/L, when the pH is 6.7.

FennoClean performic acid (PFA) is a halogen-free biocide program from Kemira. It is highly effective against primary-biofilm forming bacteria. PFA is a reaction product of formic acid and hydrogen peroxide, and its biocidal activity is based on active oxygen. PFA is thus halogen-free, corrosion safe, and also fully biodegradable. This highly efficient biocide improves paper machine cleanliness while also not generating any persistent adsorbable organic halide compounds in wastewater that could be harmful for the environment. PFA also leaves no biocidal residuals in the paper product, making it safe for end-users and the environment. Kemira PFA technology is currently available in Europe only. It is supplied with Kemira's proprietary monitoring methods to enable accurate dosing based on true needs (e.g., PiBa Assay). Table 8.28 shows features and benefits of FennoClean PFA.

Table 8.28: Features and benefits of FennoClean performic acid (PFA)

Kills microorganisms within a very short contact time
Fully biodegrading to water and carbon dioxide
Effective microbe control, provides good runnability
Cleaner felts and extended felt lifetime, improved operations and profitability
Leaves no biocide residuals in the finished product, safe for end-users
No environmental concerns
Corrosion safety, reduces machine maintenance costs
Improved environmental footprint

Based on <http://www.kemira.com/en/industries-applications/Pages/fennoclean-pfa.aspx>.

Kurita Water Industries Japan has developed a biocide sold by the name Fuzzicide. It is produced by the reaction of two chemicals—an inorganic compound with no inherent antimicrobial activity and sodium hypochlorite. Among organic slime control agents commonly used in paper mills, current methods require raising dosing concentrations to enhance antimicrobial activity, bringing to the fore the issue of proportionate increases in environmental loads. Comparatively, newly released Fuzzicide is an innovative slime control agent with high resistance to bacteria, fungi, and yeast, assuring eco-friendliness and safety. This agent has been verified to have no effect on papermaking chemicals such as dyes. Kurita Water Industries has taken the initiative in incorporating Fuzzicide into approximately 40 machines centering on fine paper for 5 years since receiving its dealership in Japan from AY Labs, an Israeli venture firm. The benefits of Fuzzicide have since been substantiated. The company further aims to expand the use of Fuzzicide in neutral-pH paper machines—such as paperboard and newsprint machines that require slime control because of changes in paper types—in addition to fine paper machines. Fuzzicide features include availability in intended water systems with high organic matter content, low metal corrosivity, and no generation of toxic waste such as trihalomethane. Fuzzicide is not supplied in containers because of easy degradation and a lack of long-term stability. It requires the installation of dedicated dosing equipment near the intended system, and the agent can be added while being constantly generated in the equipment. The dosing equipment is outfitted with a computer to control the proportions and reactions of the above two chemicals when forming Fuzzicide and to add it in appropriate quantities. Sodium hypochlorite is commonly used for sterilizing tap water. However, it tends to assign priority to dissolved organic matter rather than reacting to bacteria. Thus it is seldom used in papermaking, a process with significant dissolved organic matter that decreases antimicrobial activity. Table 8.29 shows key features of Fuzzicide.

Mills producing food packaging board use a microbial deposit control program. A multi-ply Fourdrinier board machine running at 420m/min was using a nonoxidizing microbial control program (Simons et al., 2003). A program was proposed combining a nonhalogenated oxidant with a nonoxidizing biocide. After optimization, the mill reported: spore counts in the finished paper reduced to low levels vital in food-packaging grades (average of more than 90% reduction); total aerobic microbial counts reduced to levels below mill goals (Figure 8.23); no taints, taste, or residual biocide in the final paper; improved production by 393 tons/month a cleaner machine; and improved production and provided a high-quality product. The mill also enjoyed an 854% return on investment. Another mill running under alkaline conditions at 1200m/min producing 1440 tons/day of coated wood-free paper was experiencing runnability problems

Table 8.29: Key features of Fuzzicide

Superior bactericidal effect in a short time and in small concentrations
Effective against bacteria and a wide range of microorganisms such as fungi and yeast
Little consumption by dissolved organic matter, which furthers osmosis into slime and inhibits its growth
No rise in corrosion of metals such

Based on <https://www.kurita.co.jp/english/aboutus/>.

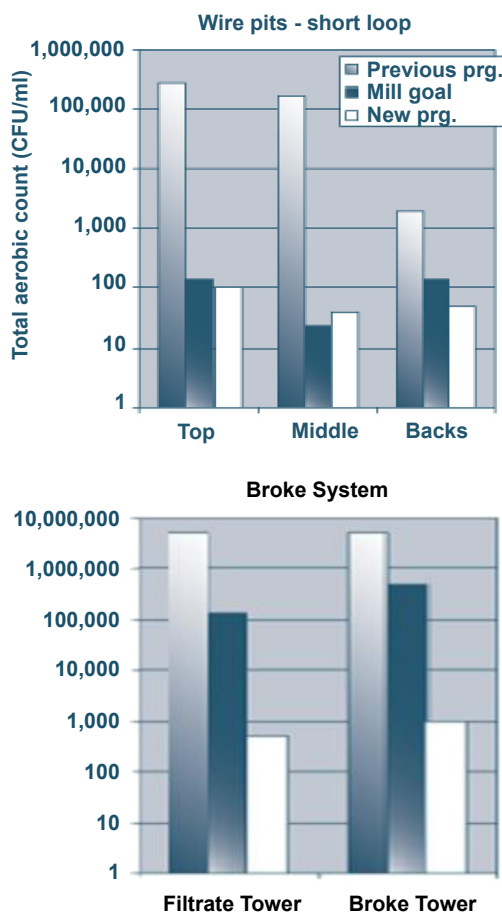


Figure 8.23

Food packaging board machine average total aerobic counts. *Simons et al. (2003). Reproduced with permission.*

because of slime buildup at the wet-end. The paper machine was using a nonoxidizing deposit control program based on MBT, DBNPA, and glutaraldehyde. The mill's fresh water was not microbiologically treated. A paper machine system survey (BioAudit) was conducted, including: understanding of system flows and volumes; system pH, temperature and oxidation-reduction potential measurements; plating studies to understand the system's microbial population; TRA-CIDE toxicity and bioactivity studies to find the best deposit control program. A program was proposed to the mill combining a nonhalogenated oxidant with a nonoxidizing biocide (*Simons et al., 2003*). The fresh water was brominated with Ondeo Nalco's ACTI-BROM to improve the control of filamentous bacteria and other microorganisms that mainly enter through this route. After startup and optimization on-site by performing TRA-CIDE cycle studies to achieve the correct feeding strategy and concentration, the mill reported the following benefits:

reel breaks reduced by 14%, from 0.53 to 0.46 breaks per day; paper holes reduced by 19%, from 2.36 to 1.89 holes per day; chemical oxygen demand(COD) decreased by 37%, from 628 to 396 mg O₂; and production increased by 3.6%, from 1152 to 1194 tons/month (Figure 8.24).

The comparisons of the performance of various biocides are presented in Table 8.30.

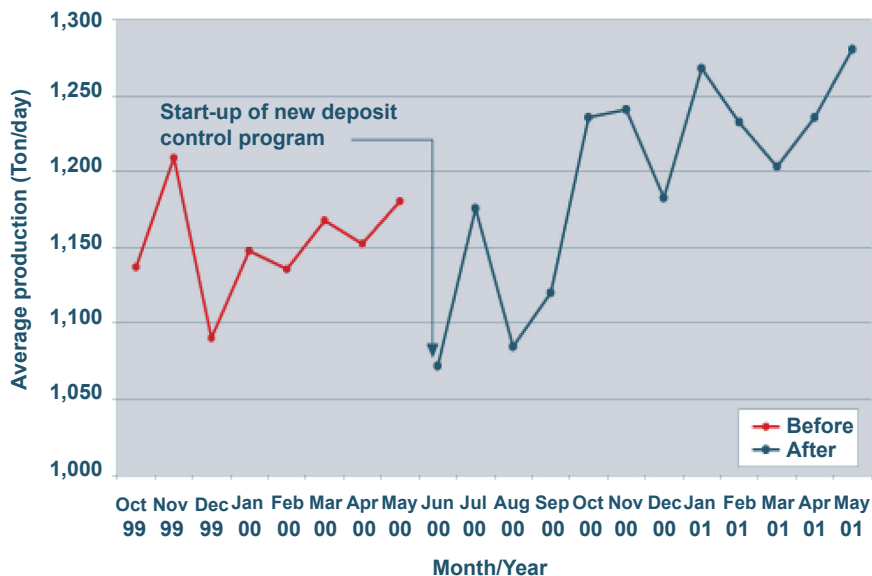


Figure 8.24

Coated wood-free paper machine monthly production counts. *Simons et al. (2003)*. Reproduced with permission.

Table 8.30: Comparison of the performance of various biocides

	Oxidizing biocides (Cl ₂ /HOCl BCDMH ClO ₂)	THPS	Glutaraldehyde	DBNPA	CMIT/MIT
Persistence	No	Yes	Yes	No	Yes
Thermal resistance	Excellent	Good	Excellent	Medium	Medium
Rate of kill	Very, very fast	Fast	Fast	Very fast	Slow
pH	4-7.5	Up to 9.5	Up to 9.5	Up to 8.5	2 to 9
Microorganisms	A, B, F	A, B	A, B, (F) biofilm +++	A, B, F biofilm ++	B, F, Y, M
Biodegradability	NA	Inherently biodegradable	Very fast	Fast	Inherently biodegradable
FA release	No	Yes	No	No	No

Based on Mirrico seminar, Kazan, September 2011.

8.4 Enzyme Use

Increasingly restrictive environmental regulations make it necessary to take the environmental issues into account in the selection of a suitable treatment. Most of the biocides conventionally employed are hazardous substances. The use of these substances is regulated by legal requirements (N.N. Directive 98/8/EC, 1998; REACH, 2006), being a potential source of pollution problems in effluents and in the environment (Blanco et al., 1996; Blanco, 2003; Johnsrud, 1997, 2000; Schenker, 1996; Schenker and Gould, 1996; Schenker et al., 1998; Van Haute, 1999; Bott, 1998). The use of substances that reduce reliance on antimicrobial agents, such as enzymes also known as “green chemicals,” would be an attractive strategy (Bajpai, 2012; Simoes et al., 2010). Enzymes have been evaluated by several researchers in both the laboratory and in paper process streams for control of microbiological slime deposits (Anonymous, 1984, 1990a; Anstey et al., 1998a,b; Fischer and Baurich, 1999; Freis, 1984; Galon, 1997; Gould, 1998; Hagelsieb et al., 1996; Hagelsieb et al., 1999; Hart, 2001; Jaquess, 1994; Kanto and Brutar, 1996; Kupfer and Baurich, 1999; Lindvall, 1998b; Siika-aho et al., 2000; Schuetz and Wollenweber, 1999; Van Haute, 1997b; Benard, 2010; Rivera and Jara, 2007; Loosvelt and Datweiler, 2007; Xu, 2005; Paice and Zhang, 2005; Buchert et al., 2004; Bajpai, 2012; Bajpai and Bajpai, 2001; Grant, 1998; Johnsrud, 1997; Torres et al., 2011, 2012; Schenker et al., 1997; Moor and Hatch, 1984; Patterson, 1986; Colasurdo and Wilton, 1988; Hatch and Moore, 1984; Augustin et al., 2004). Table 8.31 shows the characteristics and advantages of enzymatic “green” biocide.

Enzymes have been used for several years in many industries, but have had limited use and acceptance until recently in the pulp and paper industry. Some of the more common industrial enzymes are alpha amylases, cellulases, lipases, proteases, and xylanases. Enzymes contain polypeptide chains consisting of amino acids. The structure and function of enzymes are determined by the sequence of amino acids in the polypeptide chains. The molecular weights of enzymes range from around 10,000 to a million or more. The most

Table 8.31: Advantages of enzymatic biocide

Biodegradable product that does not pose any risk to mill workers or to the environment
Nonvolatile, nonreactive, and is stable during transportation
Totally replaces standard “chemical” biocides at paper mills
Shows bacteriostatic and bactericidal properties
Active against gram-positive and gram-negative bacteria
Has residual effect; it can be applied by shock loads
Does not allow bacterial strains to create microbial resistance
Eliminates biological slime in piping and equipment doing a permanent boilout
Reduces paper breaks at the PM and increases stability of the PM
Allows closure of mill water circuits without increasing water corrosivity
Reduces bad odors in the water circuits and final products

Based on Cotrino and Ordóñez (2011).

important property of enzymes is their specificity. Enzymes can catalyze one particular reaction on a particular substrate without affecting other elements. The enzyme specificity can be pictured like a lock and key where polypeptide chains fold and arrange in such a way as to form a unique binding active site for the substrate. The chemical reaction occurs at this active site.

Enzymes may affect colonization and adhesion of microorganisms in following four different ways (Longhi et al., 2008; Oulahal et al., 2007; Kristensen et al., 2008):

- Firstly, they may attack the adhesiveness of settling organisms, thus preventing the settlement event
- Second, enzymes may degrade the polymers in the biofilm matrix formed by proliferating settled organisms
- Third, enzymes may catalyze the release of antifouling compounds from the surface. These compounds may be nontoxic or toxic, but they can be much less stable than conventional biocides, what should prevent the problem of bioaccumulation of harmful chemicals
- Last, the intercellular communication during colonization of a surface may be hindered by specific enzymes

The accepted theory behind enzymatic slime control is that the enzymes degrade the extracellular polysaccharides by cleaving a specific bond in the EPS and dissolve the slime. The cells are directly exposed to the biocides once the slime gets dissolved. Biocides are now effective at lower concentration. The slime is a fructan polymer and is broken down to monomers by carbohydrase type of enzymes. Enzymes involved in the degradations of fructans are hydrolases and transferases:

Hydrolases: These are hydrolytic enzymes. According to their mode of action, they are either endo- or exo-enzymes. These enzymes produce oligofructans or only fructose.

Transferases: These enzymes split off fructose dimers and give rise to difructose anhydride by simultaneous transfructosylation.

Levan is produced by different types of bacteria, most of which are attached to surfaces. Levanase enzymes from *Streptomyces*, *Bacillus*, and *Rhodotorula* have been purified. Their molecular weights are found to be 54, 135, and 39 KDC, respectively. Levanase from *Bacillus* sp. produces levan heptose as the predominant product. It begins forming when colonizing bacteria cement to a surface and creates a biofilm by covering themselves with layers of levan polysaccharides. This creates a protective niche that completely covers the cells. It eventually grows into the large, complex slime deposits by trapping fines, fibers, mat-forming organisms, and general debris that create productivity problems for paper mills. The enzyme of *Streptomyces* sp. No. 7-3 and *Streptomyces exfoliatus* F3-2 have been found to hydrolyze levan to produce levanbiose.

The enzymatic antifouling mechanisms include the following (Torres et al., 2012; Oulahal et al., 2007; Van Houdt and Michielis, 2005; Kristensen et al., 2008; Cordeiro and Werner, 2011):

- Cell lysis by degradation of components of the cell membrane
- Degradation of compounds anchoring cells to the surface of adhesives produced during settlement and anchorage and of the extracellular matrix secreted by proliferating adhered organisms
- Disruption of intercellular communication
- Quorum sensing (i.e., bacterial cell–cell communication)
- Degradation of environmental substances that are fundamental for the survival of the fouling organisms or generating antifouling compounds

Verhoef et al. (2005) report two major approaches to develop effective enzyme products for application in the paper industry. The first approach involves directly identifying the polysaccharides present in the slime and looking for specific enzymes able to degrade them. An example of this case is levan, which is produced by several species of *Bacillus* and *Pseudomonas* bacteria that can grow in paper machines, especially those manufacturing fine paper, where the level of inhibiting compounds is low. The enzyme levan hydrolase hydrolyzes levan to low-molecular-weight polymers that are water-soluble and therefore clean the slime out of the system. Another case where it was applied is a family of products called Darazyme, developed by Grace Dearborn's group; this work was based on the preliminary identification of slime components and the subsequent application of specific enzymes or combination of enzymes depending on the polysaccharides found (Bajpai, 1999). The other approach is indirectly testing to identify enzymes that show activity on the biofilm by studying their effects on slime development. The specificity in the way enzymes interact with the biofilm makes this a complex technique, being somewhat difficult to identify enzymes that are effective against different types of slimes. According to Simoes et al. (2010), formulations containing several types of enzymes seem to be fundamental when it comes to applying this slime control approach successfully.

The use of enzymes in the control of microbiological slime deposits has proved useful under modern mill conditions (Hatcher, 1983; Grussenmeyer and Wollenweber, 1992, 1993). EDC-I (enzymatic deposit control) is an enzyme patented by Economics Laboratory (Hatcher, 1973) that hydrolyzes and depolymerizes the fructose polysaccharide levan, which has been identified in paper mill slime (Colasurdo and Wilton, 1988). The product is environmentally safe and has no effect on the operation of activated sludge effluent plants. EDC-I has been successfully applied in systems with wide range of temperature and pH. It is used by paper mills in the United Kingdom, Scandinavia, Japan, and the United States. Applications have been on machines producing writing and printing, fine paper grades, and paperboards. This enzyme is produced during an aerobic fermentation of a common nonpathogenic, non-spore-forming soil bacterium. Personal safety is greatly enhanced through the use of an enzyme product

rather than conventional toxic biocides. The enzyme is nontoxic, does not release dangerous fumes and is not corrosive and also does not require any special handling. Spills can be flushed away with water. The exact period for which the enzyme is stable depends on a number of factors peculiar to any paper machine system, including the temperature and pH profiles as well as any other additive.

Leroy et al. (2008) and Molobela et al. (2010) have reported that proteases, and particularly serine proteases, are most efficient in removing *Pseudoalteromonas* and *Pseudomonas fluorescens* biofilms. Lequette et al. (2010) showed that serine proteases were efficient in removing *Bacillus cereus* biofilms, whereas glycosidases were efficient on *P. fluorescens* biofilms when used as a mix of α - and β -polysaccharidases. Marcato-Romain et al. (2012) characterized biofilms from the paper industry and evaluated the effectiveness of enzymatic treatments in reducing them. The EPS extracted from six industrial biofilms were studied. EPS were mainly proteins, the protein to polysaccharide ratio ranged from 1.3 to 8.6 depending on where the sampling point was situated in the papermaking process. Eight hydrolytic enzymes were screened on a 24-h multispecies biofilm. The enzymes were tested at various concentrations and contact durations. Glycosidases and lipases were inefficient or only slightly efficient for biofilm reduction, whereas proteases were more efficient: after treatment for 24 h with pepsin, Alcalase1, or Savinase1, the removal exceeded 80%. Savinase1 was tested on an industrial biofilm sample. This enzyme led to a significant release of proteins from the EPS matrix, indicating its potential efficiency on an industrial scale.

Torres et al. (2011) studied the effectiveness of 17 commercial enzymatic products on biofilm formed by the flora present in the process water obtained in the sheet forming zone of the wire section from a 100% recycling paper mill using mixed recovered paper as raw material and producing paper for board. The results showed that Pectinex Smash and its fraction Novoshape were the best formulations in the prevention of biofilm formation (Blanco et al., 2011). Pectinex Smash® has a mixture of pectinolytic activities and Novoshape is an enzymatic solution of a microbial pectin methylesterase. The gene encoding of the esterase enzyme is derived from fungus *Abdopus aculeatus* and is transferred into a strain of the food grade organism *Aspergillus oryzae* for commercial production. The Novoshape preparation has a declared activity of 10 PEU/mL and an optimal temperature of 50 °C. This enzyme belongs to the carbohydrate esterase family, catalyzes the hydrolysis of methyl ester groups, and has high specificity for pectin substrates, a property widely used in food industry and in plant science.

The combination treatment of enzyme and selected biocides has a substantial effect on cell survival. Ferris et al. (1989) reported that in all cases, the combination treatment resulted in significantly greater population reduction compared with the use of biocide alone at the same concentration. The enzyme alone showed no effects. Further, it was shown that on

application of enzyme (0.10 kg/metric ton) on machines producing printing grade paper, the biocide concentration could be reduced from 0.15 to 0.02 kg/metric ton (Ferris et al., 1989). The amount of biocide was reduced to ~15% of what had been used before using the enzyme. Hatcher (1983) also achieved a similar level of reduction in biocide concentration in the whitewater system of a paper mill when an enzyme preparation was used. Patterson (1986) has reported that with the use of enzyme, the concentration of biocide may be reduced by 50% and slime breaks are reduced from three per day to three per month. Colasurdo and Wilton (1988) reported that with the use of an enzyme preparation at Sonoco Products Co., Hartsville, Georgia, the slime breaks were almost eliminated which resulted in increased productivity. The Levanase enzyme produced by *Rhodotorula* sp. has been found to reduce the required biocide concentration by 25% without negatively affecting the paper properties (Chaudhary, 1992; Chaudhary et al., 1998). In spite of the strong indications that exopolysaccharides are involved in adhesion, enzyme treatment has shown that proteases are more effective than carbohydrate-degrading enzymes in removing bacteria from surfaces.

Microorganisms have cell walls, which give rigidity. If the cell wall is removed in some manner, the cell usually dies because of lysis, which results from osmotic balance. In microorganisms, the cell walls contain substances like cellulose, chitin, mucopeptide, and β -1,3-glucan. In addition to these, many microorganisms have capsules, slime layers, or other surface components, which are polysaccharides or proteins attached to the outside of the rigid layer conferring additional rigidity and/or protection. There are enzymes which will attack all of these polymers. The use of lytic enzymes with gluconase and protease activity in paper machine process water to kill microorganisms causing precipitates and/or forming slime and/or adversely affecting product quality, has been promoted. Enzymatic dispersion of biological slimes has been studied with pentosanase-hexosanase enzyme, Rhodozyme HP-150. This enzyme is especially effective in treating industrial waters used in the operation of cooling towers to disperse slimes and slime-forming masses within such waters to prevent the deposit of such slimes on the heat exchange surfaces of cooling towers and surface associated with such units. In his patent, Orndorff (1983) has described a method of killing and inhibiting the growth of microorganisms in industrial process streams using peroxidase- or lacasse-catalyzed oxidation of various phenolic compounds to generate microbial oxidation products (e.g., poisonous quinines).

Enzyme-based antislime—Betzdearborn's Spectrum—has been developed in response to such pressures of the European biocide directive ecolabeling, ISO 1400, and mill water system closure. This is available in the market and among its tool kit of products is a gluconase enzyme, which catalyzes the attack of glucose, which represents a significant proportion of the polysaccharides in the EPS on paper machines. In addition, formation of this troublesome polysaccharide coating is inhibited by new and nonenzymatic materials in Spectrum, which interfere with slime formation mechanism (Bajpai, 2012).

The use of a mixture of enzymes has been found to be more effective in treating microbially produced extracellular polysaccharides in cooling water and in papermaking broke water than the use of a single enzyme. The composite enzyme system tested consisted of cellulase, alpha amylase, and protease. According to some manufacturers and service companies, the enzymes available are too species-specific and can be used only on a smaller scale or as a supplement to conventional biocidal methods.

Weyerhaeuser Inc., Dryden, Ontario, a fine paper mill in Canada, put an increased emphasis on paper machine shutdown cleaning and deposit control. This helped the mill to substantially reduce breaks and increase efficiency (Daignault and Jones, 2003). A coordinated chemical deposit-control program was supplemented by cleaning programs focused on difficult to reach areas that were suspected as possible contributors to breaks. The big breakthrough in starch system cleaning has been the introduction of enzyme-cleaning products. The enzyme used is amylase, an enzyme that breaks down the starch deposits and thus allowing them to be easily washed away. The enzyme is so powerful and persistent that it is necessary to deactivate the enzyme with hypochlorite or peroxide after the cleaning. The Dryden mill has used the starch enzymatic cleaner for the past few years with very good success. In the early enzymatic starch cleanings, the system was found to have leaks after the enzyme boilout. Starch deposits that had plugged the leaks were completely cleaned away. In addition to the enzymatic cleaning, regular caustic cleanings of the system were done. These were found to be effective but do not match the enzyme cleaning. The size press area itself is cleaned with hot water.

The lytic activity of biological catalyst enzymes reportedly prevents biofilm formation. Steve patented a method that implemented plant dehydrogenase enzymes such as peroxidase and laccase to kill or inhibit the growth of microorganisms in industrial processes (Steve, 1983). Similarly, studies were proposed to use microbial or plant enzymes in the presence of oxidants to degrade the bacterially produced polysaccharides in biofilms (Ratto et al., 2001; Jedrzejewski, 2000). A new group of biocides based on the cell wall lytic enzymes obtained from microorganisms, such as β -1,3-glucanases, chitinases, and proteases, attack bacterial, fungal, and yeast cell walls (Stepnaya et al., 2008; Shastry and Prasad, 2005; Bar-Shimon et al., 2004; Nagaraj Kumar et al., 2004; Brito et al., 1989). Tables 8.32–8.34 show the results of applying the new enzymatic biocide at pulp and paper mills (Cottrino and Ordonez, 2011).

Klahre et al. (1998), however, report poor performance of enzymes alone in antifouling efforts in paper mills, particularly in long-term applications. The enzymes themselves are rapidly degraded by extracellular proteases. Brisou (1995) already showed that there were a vast variety of target structures that enzymes had to interact with, indicating that there is no single enzyme or enzyme mixture to effectively remove biofilms.

The specificity in the enzymes mode of action makes it a complex technique, increasing the difficulty of identifying enzymes that are effective against all the different types of biofilms.

Table 8.32: Bacterial control at a tissue paper mill starting the use of the enzymatic biocide

Day	Bacterial count at wire pit (cfu/g)
0	60 million
5	15 million
10	4–5 million

Based on [Cotrino and Ordóñez \(2011\)](#).

Table 8.33: Downtime reduction because of removal of dirt and detachment of slime at paper machine using enzymatic biocide at an OCC mill

1. *Downtime reduction because of elimination of dirt detachment*
 - a. Reduction from 5.6 h/day to 0.4 h/day on PM 1 (reduction 93%)
 - b. Reduction from 2.7 h/day to 0.2 h/day on PM 2 (reduction 92%)
2. *Downtime reduction because of elimination of slime detachment*
 - a. Reduction from 0.5 h/day to 0.1 h/day on PM 1 (reduction 74%)
 - b. Reduction from 0.8 h/day to 0.3 h/day on PM 2 (reduction 70%)

Based on [Cotrino and Ordóñez \(2011\)](#).

Table 8.34: Bacterial count at the machine chest of an OCC recycling mill using the enzymatic biocide. Monthly average values of total bacterial count at the machine chest in an OCC recycling mill

Month	Bacterial count (cfu/mL $\times 10^6$)
Initial	21
April	9
May	7
June	7
July	6.8
August	5.69

Based on [Cotrino and Ordóñez \(2011\)](#).

Formulations containing several different enzymes seem to be fundamental for a successful biofilm control strategy. Basically, proteases, and polysaccharide hydrolyzing enzymes may be useful ([Meyer, 2003](#)). Moreover, the use of enzymes in biofilm control is still limited because of the low prices of the chemicals used today compared with the costs of the enzymes. In fact, the technology and production of these enzymes and the enzyme-based detergents are mostly patent-protected. Moreover, the low commercial accessibility of different enzyme activities limits their current usage ([Johansen et al., 1997](#)).

[Orgaz et al. \(2006\)](#) in their study on biofilm removal, concluded that on biofilm removal of *P. fluorescens*, the enzyme pectin esterase produced by *Trichoderma viride* (which belongs to the same family as pectinase methylesterase), could possibly deacetylate a polysaccharide in the biofilm matrix, making it softer and possibly more porous ([Orgaz et al., 2006, 2007](#)). Many microbial EPS have different substituent groups as ketal-linked pyruvate or ester-linked

acetyl groups. The removal of acyl groups, especially the acetate, can significantly affect the physical properties of polysaccharides. The removal of one of the substituents such as the acetyl group, in particular the acetate, influences the physical properties of EPS (Sutherland, 1999). Several fungi can degrade complex plant cell-wall material, by secreting a large variety of enzymes. This versatility makes commercial polysaccharide-degrading enzyme mixtures have a widespread use in multiple fields, such as fruit processing (McKay, 1993) or wastewater treatment (Wesenberg et al., 2003). They could also be used to degrade bacterial biofilm matrices or prevent and control the formation of biofilm in the piping system of the paper mill wastewater. Because of the EPS heterogeneity, a mixture of enzymes might be necessary for efficient biofilm degradation.

8.5 Biological Equilibrium

Biological equilibrium allows biocide-free operation and is applicable to all paper grades. This process is based on the principle of natural biological equilibrium. In this process, nontoxic, modified lignosulfonates are used with specific components that are chelating agent different from those generally used in papermaking. The lignosulfonate is water soluble. With controlled bacterial activity, efficient system cleaning and the reduction of chemical deposits is obtained. This process maintains the organisms and the suspended matter in the whitewater in a colloidal state and prevents the bacteria from forming deposits and agglomerating. The bacteria adheres to the modified lignosulfonate. It carries a net negative charge. The material becomes more flocculent as the bacteria digest the organic substances in the colloids. The lignosulfonate is drained off on the wire press and the bacteria enter the finished paper. Counterindications are open water systems, use of recycled fibers, unbleached kraft pulp, and an increase in the organic matter (Oberkofler, 1987, 1989, 1992; Oberkofler and Braunsperger, 1994; Braunsperger et al., 1996).

BIM Kemi AB has marketed biocide-free slime control since 1981, and its own product, Bimogard, since 1991 (Anonymous, 1986; Bjorklund, 1999, 2000, 2001a,b; Bjorklund, 2002a,b; Gavelin, 1996). Bimogard is a multifunctional, multicomponent product consisting of chemically modified lignosulfonates and is fortified with various surfactants. It is not toxic to microorganisms at concentrations used in mills. It is the first completely biocide-free slime control system in Europe. This process has been successfully used in low as well as neutral to high pH conditions, and at small, old, as well as large, modern, fast-moving paper machines. It is also used as intermittent board machines in pulp mills. The Bimogard system appears similar to the Biochem Process (Morros, 1995). This product is based on modified lignins and surfactants. It functions by:

- Cleaning the surfaces and thus reducing the adhesion of bacteria
- Reducing microorganism activity by inhibiting the production of extracellular polysaccharides
- Reducing bacterial growth and spore formation

Several Bimogard systems are in operation on paper machines for lightweight coated fine paper, newsprint, tissue, greaseproof paper, and paperboard. Results show that the system has brought about considerable improvements in the efficiency:

- Hole frequency declines
- Time between washups increases
- Cleaning becomes easier
- Lesser breaks in the wet-end; lesser production of broke
- Cost for using Bimogard for slime control is the same or most often less than using biocides
- Bacterial count is usually 10^5 – 10^7 /mL in mills using Bimogard. Most of these bacteria do not cause any harm and die in the drying section without causing slime problems
- Does not trigger bacterial defense mechanisms unlike biocides

Bjorklund (2001b) studied the effect of Bimogard on the amount of EPS after introduction to a mill before using biocides. The amount of EPS decreased after introduction of biocide-free slime control in paper mill using 100% deinked pulp (DIP).

Bimogard has many other advantages (Table 8.35). Table 8.36 shows the effect of Bimogard on the amount of EPS after introduction to a mill previously using biocides. It can be clearly seen that the amount of EPS decreases after introduction of biocide-free slime control in paper mill using 100% DIP. The bacteria count is usually 10^5 – 10^7 /mL in mills using Bimogard. Most of these bacteria are harmless and die in the drying section without causing slime problems.

Bimogard is currently running on several machines in Scandinavia using a variety of different pulps for production of tissue, newsprint, and carton as seen in Table 8.37. In Sweden, this has decreased the use of toxic slimicides with 20–25%. Also, this slime control has been successfully used in low as well as neutral to high pH conditions, and at small, old as well as large, modern, fast-moving paper machines. Bimogard is also used at intermittent board machines in pulp mills.

Table 8.35: Advantages of Bimogard

Keeps surfaces clean and smooth thereby preventing biofouling
Decreases the production of EPS (“slime”), thereby preventing growth of biofilm
Delays the growth of bacteria and decreases the formation of spores, thereby preventing bacteria in ready-made paper or carton
pH-stable
Temperature-stable
Environmentally friendly
Does not endanger the health of the staff
Is proven to be a satisfactory or even better alternative to biocides
Is cost-effective

Based on Bjorklund M (2001b).

Table 8.36: Effect of Bimogard on EPS after introduction to a mill previously using biocides

Sample	EPS (mg/g)			
	Before introduction of Bimogard	2 weeks after introduction of Bimogard	4 weeks after introduction of Bimogard	16 weeks after introduction of Bimogard
Deposit from section under forming roll	13.0	6.1	5.5	0.0
Back water	6.3	3.1	1.2	0.0
Process water from wire pit	3.8	1.3	0.0	0.0
Fresh water	0.0	0.0	0.0	0.0

Based on Bjorklund M (2001b).

Table 8.37: Mills using Bimogard

Paper grade	Type of pulp
Tissue	Sulfate
Tissue	DIP
Tissue	DIP
Tissue	DIP
Tissue	Sulfate
Tissue	Sulfite, sulfate
Printing and writing paper	Sulfite, sulfate
Printing and writing paper	Sulfite, sulfate
Greaseproof	Sulfate
Newsprint	DIP, TMP
White-lined chipboard	Waste paper
Tissue	Sulfate
Newsprint	DIP, TMP
Newsprint	DIP, TMP
Newsprint	DIP, TMP
Newsprint	DIP, TMP
Wood-containing printing paper	Sulfite, SGW-pulp
Folding box board	SGW-pulp, sulfate
Printing and writing paper	Sulfite
Printing and writing paper	Sulfite

Based on Bjorklund M (2001b).

A method for closed water systems from Petromontan uses a multifractionated and modified ligno-sulfate called Petrodis (Anonymous, 1986). This reduces the requirement of biocides when added to the system and also results in better functioning of the paper machine. This is attributed to:

- More efficient biological control
- Reduced chemical scaling

- Reduction of toxic substances in the system and in the effluent
- Safer for workers
- Reduces costs for the control of slime and scale

8.6 Biodispersants

Biofilm control programs can be made more effective through the use of biopenetrant/dispersant products. The use of biodispersants offers an ecologically attractive method of controlling slime in the pulp and paper industry. They eliminate or reduce the needs for biocides (Anstey et al., 1998a; Blankenburg and Schulte, 1997, 1999; Gould, 1998, 2001; Pauly, 2001; Robertson and Taylor, 1994; Saner, 1998; Stenqvist, 1992; Van Haute, 1997a,b; Van Haute, 1999; Weissshuhn et al., 2000; Wright, 1997; Schenker, 1996). These are nonbio-cidal surface-active agents that penetrate and loosen the biopolymer matrix. This not only aids in sloughing the biofilm, but will also expose the microorganisms to the microbicides. They can be continuously applied or periodically slug-dosed. Because these products effectively mobilize solids, they can cause clogging in downstream and down-gradient locations, and this potential must be taken into account when designing proper application. Biodispersants are particularly effective when dealing with systems that have a high total organic carbon loading and a tendency to foul. These products are typically fed in slug additions before microbicide feed. Low-level continuous feed may also be effective. However, in some cases, this may not be as effective because it may not reach a certain threshold amount required to produce the desired effect. Developments in biodispersant technology are making this approach more effective and popular than ever before. This technology is based on nonionic polymers, which are nontoxic, nonfoaming, colorless, and free of organic solvents. Because of their nonionic character, they will not increase the system anionicity and also not interfere with other papermaking chemicals. Table 8.38 shows biodispersants used in the paper industry.

Biodispersants dissolve and disperse deposits, preventing the biofilm from reestablishing itself. These dispersants act as biopenetrants opening the biofilms and allowing biocides to penetrate the exopolysaccharides. It has been also reported that dispersants facilitate

Table 8.38: Biodispersants used in the paper industry

Synonym	Chemical basis
Amides	Alkylamides
Cationic, hybrid ionic	Fatty acids
Glycols	Alcohols
Lignosulfonates	Salts of lignosulfonic acid
Non ionic, anionic	Alcohol
Terpenes	Terpenoids

Based on Schrijver and Wirth (2007).

penetration of biocides into the cell or that they trigger sloughing of biofilms. Biodispersants have no pH limitations and are suitable for use in both acidic and alkaline papermaking.

Surface-active agents for slime control may be prepared by chemical synthesis, obtained as a byproduct of industrial processes (e.g., lignosulfonates), manufactured from natural raw materials (e.g., paraffin wax, terpenes from oranges, produced by cultivated microorganisms (Johnsrud, 1997)). The industrial dispersants can be divided in three categories:

- Anionic dispersants: alkyl and aryl sulfonates are the most widely used dispersants of this group. They consist of benzene sulfonic acids with alkyl chains of 10–15 carbon atoms in the para position (Atwood and Florence, 1983). These surfactants can be used as the sodium salt or in conjunction with other surfactants (Blanco, 2003).
- Cationic dispersants: some of the chemicals, used commonly as cationic surfactants can also have biocidal properties (Blanco, 2003). An example of this combined action is that of quaternary ammonium compounds.
- Nonionic dispersants: examples of this group of surfactants are n-octyl glucoside and polyethylene oxide (10) cetyl ether (Blanco, 2003).

The nonionic products, which have seen large-scale commercial use, are of the four types—alcohol ethoxylates, alkylphenol ethoxylates, polyoxyethylene esters, and polyoxyethylene-polyoxypropylene derivatives. The latter are mixed polymers with hydrophobic groups derived from propylene oxide, further reacted with ethylene oxide until the desired properties are achieved. Generally, they are of rather high molecular mass, often with much more than the usual eight to 15 molecules of ethylene oxide characteristics of the other nonionics. Dispersants do not kill or do not always even inhibit the growth of microorganisms. Therefore, their dosage or evaluation of their effect cannot be based on cell counts in circulation waters and new efficient methods to evaluate their efficiency in mill conditions are required (Blanco, 2003).

The chemistry of dispersing agents can be designed to give various dispersing/solubilizing and emulsifying properties to the product, which may disturb the biofilm, stabilize the emulsion with cells and biofilm components, and form a protective layer on the hydrophobic surfaces that delays the attachment of biofilm (Blankenburg and Schulte, 1996). Different dispersants chemistries can be designed according to the propose use (Kanto et al., 2001).

The use of organic natural products has reported a few problems such as the high concentrations required and the microorganisms may use the natural products as a nutrient source (Blanco, 2003). For example, lignosulfonates can increase the growth of microorganisms under certain conditions because of the presence of sugars that can be used as nutrients by the microorganisms (Cardoso, 1992; Robertson and Taylor, 1994).

The dosage and design of the treatment system depends on the problem to be solved. Recommended addition points are the short circuit before the headbox or to clarified water (Johnsrud, 1997). The dispersants treatments may be applied either to obtain biocide-free microbial control programs or in combination with biocide in order to reduce the dosage needed (Schenker, 1996).

The mechanism by which conventional dispersant additives function in papermaking systems is by contributing a high negative ionic charge to the surfaces upon which they get adsorbed (Ashraf et al., 2014). The increased negative charge of the particle surfaces increases the electrostatic potential energy barrier and reduces contact with the surfaces because the bacteria are negatively charged. To be effective, the amount of dispersant must be sufficient to overpower any coagulants that may be present (e.g., aluminum sulfate, high-density cationic polymers). There are some problems with the use of dispersants. These products effectively mobilize solids but they can cause clogging in downstream and down-gradient locations; therefore, this potential must be considered when designing an appropriate application that will not lead to an uncontrolled accumulation of dispersed materials in a paper production system. Particularly cationic retention aids tend to be deactivated by dispersants. However, in some cases, dispersants have helped papermakers overcome specific deposit problems (Hubbe et al., 2006). Under some circumstances, slime deposits may be responsible for up to 70% of all breakages, blockages, and pump failures in pulp and papers mills (Safade, 1988).

A deposit control program was proposed to a paper mill producing uncoated wood-free and specialty papers with the goal of reducing the use of biocides while maintaining or improving the overall performance at similar costs (Simons et al., 2003). The recommended program involved the continuous application of a biodispersant while reducing feeding frequency of the same nonoxidizing biocides used previously by the mill. After optimization, the mill reported reduced nonoxidizing biocide use by 60%, fewer deposits on the paper machine, sheet breaks reduced from an average of 5.8–2.6 breaks per day, and deposit control program costs reduced by 20%. This demonstrates that selected dispersants can significantly reduce the amount of nonoxidizing biocides needed to obtain good slime control in a cost-effective manner. Poor microbial deposit control is the main cause of poor paper machine runnability and loss of efficiency. The use of new monitoring tools allows mills to optimize slime control programs to achieve the best performance in a cost-effective way. The use of traditional toxic nonoxidizing biocides can be significantly reduced by the use of new nonhalogenated oxidants in combination with new nontoxic deposit control agents. An optimized microbial deposit control program can give papermakers a high return on investment, because program costs can be offset by improved runnability, decreased rejects, and reduced biological oxygen demand (BOD) in the final effluent.

A new biofilm cleaner has been developed that penetrates and destroys biofilms (<http://www.industrialchemtex.com/docarchive/pub/TT-32%20Biofilms.pdf>). This technology destroys the biopolymer matrix, but it also attacks the attachment structures and completely removes the biomass from the metal surface. The penetrating action of the cleaner exposes the microorganisms growing there to the effects of the microbicide being used. This will reduce the amount of biocide required to achieve a complete kill of algae and bacteria in the system. By leaving a bare metal surface, it also allows the corrosion inhibitor to reestablish protection. The biofilm cleaner should be slug-fed to a point in the system where good mixing is

obtained. Addition should be made 30 min to 1 h before biocide addition. The level of cleaner required will be dependent on the amount of biofilm present. As with the biocides themselves, frequency of addition depends on the rate of reinfection, the level of nutrients available, and the ambient conditions.

Buckman Laboratories has developed new technologies to control biological deposits for more than 50 years. Buckman researchers, microbiologists, and application engineers have developed the Neoteric Product Line for deposit control. Neoteric biodispersants are new, specially designed Buckman products for use in preventing and slowing biofilm development in paper machine systems without sacrificing papermaking operations. These products can penetrate existing biofilms, loosening and dispensing them, which allows biocides to penetrate and reach the organisms, and were developed with safety and the environment in mind (Van Haute, 1999; Hart, 2001; Koopmans and de Vreese, 2002). The programs have been successfully used in acidic, neutral, and alkaline conditions and in the manufacture of all types of paper, paperboard, tissue, and toweling (Van Haute, 1999, 2000). Buckman reports that several paper machines in Europe use Neoterics in their short circulation loop, with Busperse and Buzyme. Neoteric programs are designed to increase the effectiveness of traditional biocides, reducing and in some cases eliminating the use of biocides in wet-end. Neoteric programs consist of dispersants, enzymes, and potentiators added to the wet-end of the paper machine:

- Neoteric dispersants are a select group of chemicals that prevent agglomeration and deposition on machine surfaces of common deposit causing components in the paper machine furnish.
- Neoteric enzymes are biodegradable and nonbiocidal, have relatively low level of toxicity to test animals, and are Food and Drug Administration–allowed. These enzymes include amylase enzymes and protease enzymes in a formulation that attacks starches and proteins.
- Potentiators are nonbiocidal products that increase the performance of biocide. These products allow the use of less organic biocide while still achieving the same effect as the original dosage of biocide. The potentiators simply lower the effective concentration of biocide required for a given application. These products, when used alone, do not reduce or slow microbial growth or metabolism.

Neoteric deposit control helps to improve sheet quality, reducing holes, breaks, and off-specification production. It has no negative impact on sizing, color, retention, drainage, or other machine operations when used as directed. Neoteric deposit control can help reduce lost production by keeping machines running longer between washups and can provide better utilization of resources such as energy, water, and manpower. Jacquelin et al. (1994) also reported the synergistic action of enzymes in combination with surfactants and phenolic antimicrobials. The combination of proteolytic enzymes with surfactants was found to enhance the efficiency of cleaning on *Bacillus* spp. biofilms (Parkar et al., 2004).

Nalco recommends lignosulfonate-based biodispersants plus thorough machinery and system cleansing; elimination of static areas and rough surfaces; fresh water and additive contamination controls; and good retention and prevention of anaerobic bacteria in storage tanks through oxygenation for closed paper machine systems. The name biodispersant suggests the activity on a biological entity, but laboratory study has shown that many commercial biodispersants, in the absence of a microbicide, have little or no ability to remove an existing biofilm or prevent their formation. In fact, most of the commercial biodispersant applications include the use of a microbicide. Biodispersant technology can increase the overall efficiency of a given microbicide program.

Buckman Laboratories have developed an enzyme-based product line of biodispersants for boilouts and other applications ([Wolfanger, 2001](#)). This patented enzyme stabilization process promotes greater shelf life and reduced temperature instability concerns. It also reduces significant safety and health hazards in paper machine operations that are present during caustic boilout. It is also possible to release nontoxic cleaning solutions without any treatment before discharge. These products were found to be successful in mill operation. A fine, coated paper mill wanted to enhance safety by eliminating the use of caustic during traditional paper machine boilouts. Buckman began an enzyme-based Neoteric boilout program that matched the effectiveness of the traditional boilout and in some areas—especially the headbox—exceeded it. The mill now completes boilouts in less time and has a cleaner system, with fewer related startup deposits than experienced with caustic boilouts. Another fine paper mill using softwood and hardwood kraft furnish had a problem with starch deposits in its size press system. Buckman replaced their caustic boilout with its Neoteric program that quickly and completely removed the starch deposits. Another mill producing 100% recycled paperboard was concerned about caustic safety issues and had a problem with paper machine system deposits. Buckman's Neoteric enzymatic boilout program eliminated deposits in tanks and lines and reduced safety and health concerns.

Enzyme-based boilouts remove the compounds such as starch, slime, pitch, adhesives, latex, and other synthetic binders that hold the deposits together. The type of enzyme used and the dispersant depends on the type and amount of deposit present in the system. Starch slurry contains deposits that are microbiological and/or starch protein based. Boilouts using a product that contains a stabilized protease enzyme are found to be effective in these systems. For the cooked starch system, an alpha amylase product is used to remove deposits comprised mainly of cooked starch. In both cases, a preboilout system flush is essential. This removes the cooked starch and allows the enzyme to work particularly on deposits. An ideal starch system boilout uses the following:

- 0.2–0.5% of the boilout product
- Temperature of 120–150 °F
- Recirculation time of 1–2 h

Enzymatic boilouts are pH neutral and can be dumped directly to the sewer without neutralizing the solution and without any upset at the waste treatment facility. Also, it removes the safety concerns associated with working with and around the caustic solution. It eliminates the requirement for excessive rinsing to purge caustic from the system. Each of these factors contributes to a reduction in the downtime necessary for a boilout and maintenance outage, which shows cost savings.

Starch-based coating systems can be successfully cleaned via a boilout using the alpha amylase enzyme. The parameters for this boilout are similar with the enzyme-based boilout product as mentioned previously. It is required to use a neutral dispersant/penetrant capable of removing latex and other binders that may be present in the coating formulation in addition to the enzyme-based product. Also a higher temperature range (130–150 °F) and a longer recirculation time (2h) will be beneficial for a starch-based coating system boilout. Enzyme boilouts are not recommended for the coating systems that do not contain starch. Enzyme boilouts have been successfully applied in systems that use both starch-based and non-starch-based coatings. Enzyme-based chemistries are found to be successful for different types of paper mills ranging from acid to alkaline papermaking, recycled to virgin, board to fine paper to tissue, including all variety of additives linked with these various furnishes. The question arises about how this is possible given the specific mode of action of enzymes. This appears logical, if the majority of the machine system deposits are microbiological slime. A protease breaks down these deposits. But in a system that has deposits containing various fiber, fillers, and hydrolyzed additives, how does the protease enzyme work? Actually, most deposits in the paper machine system have microbiological growth in, on, and around the deposit. When the enzyme breaks down this matrix, the deposit sloughs off and is carried away in the boilout solution. This is based on the ability of bioengineered enzymatics to attack specific deposits by eating the organisms that compose them, making them easy to rinse away. There are certain pH-neutral additives that can be added to help in penetrating, breaking down, and removing deposits that are nonmicrobiological in nature. These additives in fact help the protease-based enzyme product enter into the deposit where microbiological growth is helping to anchor and hold a deposit together. This is a cleaning effect and is found to be better than caustic boilouts.

Paper companies are currently striving toward environmental stewardship, worker health, and safety and producing a product of the highest quality. Neutral boilouts are a major step forward in application technology to help the papermaker in meeting these difficult objectives. The future is sure to bring new and varied use of enzyme technology to further benefit the paper industry through the further development of stabilized enzyme products.

8.7 Use of Competing Microorganisms

Some nonconventional approaches have been used in solving slime problems. The literature shows the existence of multiple interspecies interactions or the simple production of a

metabolite can interfere with biofilm formation and development (Carpentier and Chassing, 2004; Kives et al., 2005; Røssland et al., 2005; Tait and Sutherland, 1998; Valle et al., 2006). Competition for substrates is one of the major evolutionary driving forces in the bacterial world, and several experimental data obtained in the laboratory, under controlled conditions, show how different microorganisms may effectively outcompete others because of their better utilization of a given energy source (Christensen et al., 2002; Simoes et al., 2007; Lindvall, 1998a).

The inoculation of non-slime-forming organisms to outcompete the slime formers has been proposed. A slime control process has been patented by Oberkofler (1993). This is based on the introduction of a consortium of bacteria commercially available in freeze-dried or liquid form. The bacteria are pregrown before inoculation of the circuit water, and the amount added is calculated on the basis of total organic carbon present. The additives may be introduced along with the bacteria, thus favoring their growth. These additives include tensides to avoid the adhesion of bacteria, lignosulfonate to increase the nutrients, and enzymes to catalyze the breakdown of organic substances. It has been also suggested aeration of the circulating water with oxygen or air or the addition of oxygen releasing compounds such as hydrogen peroxide. The invention is not limited to bacteria, but the fungi alone or the mixture of fungi and bacteria can also be used. The patent describes an indiscriminate use of bacteria and fungi, of which the mixed culture of freeze-dried bacteria used contains genera associated with slime formation in pulp and paper mills but also contains such genera undesirable for paper and board intended to come into contact with food stuffs. Plant-scale trials showed that the addition of the selected microbes to the circulating water reduced the buildup of slime on solid surfaces and in the liquid phase.

Many bacteria synthesize and excrete biosurfactants with antiadhesive properties (Desai and Banat, 1997; Nitschke and Costa, 2007; Rodrigues et al., 2004; van Hamme et al., 2006). Rodrigues et al. (2004) reported that biosurfactants produced by *Lactococcus lactis* 53 impaired biofilm formation on silicone rubber. Surfactin from *B. subtilis* breaks biofilms without affecting cell growth and prevents biofilm formation by microbes such as *Salmonella enterica*, *E. coli*, and *Proteus mirabilis* (Mireles et al., 2001). Other biosurfactants showed biofilm control potential (Davey et al., 2003; Walencka et al., 2008; Rivardo et al., 2009). Microbial molecules, used as biopreservatives, such as nisin, lauricidin, reuterin, and pediocin, have been reported for their biofilm control potential against microbes generally found in dairy processing facilities, including *Listeria monocytogenes* (Dufour et al., 2004; Garcia-Almendarez et al., 2008; Mahdavi et al., 2007; Zhao et al., 2004). Valle et al. (2006) showed that *E. coli* expressing group II capsules release a soluble polysaccharide into their environment that induces physicochemical surface alterations, which prevent formation of biofilm by gram-positive and gram-negative bacteria. Davies and Marques (2009) observed that *P. aeruginosa* produces *cis*-2- decenoic acid, which induces the dispersion of established biofilms and of inhibiting biofilm development. This molecule was tested, when applied exogenously, against *B. subtilis*, *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa*,

P. mirabilis, *Streptococcus pyogenes*, and the yeast *C. albicans*. It has been suggested that this molecule is functionally and structurally related to the class of short-chain fatty acid signalling molecules.

8.8 Biofilm Inhibitors

Biofilm inhibitors prevent biofilm formation at an earlier stage than biodispersants and enzymes and are an effective environment friendly alternative treatment for short loops. Sulfosuccinates, a family of molecules, are very effective in inhibiting deposit formation (Davis et al., 1999; Scharpf, 1998; Schenker et al., 1998). An exclusively designed sulfosuccinate molecule is found to act at an earlier stage than biodispersants and enzymes in the inhibition of biofilm deposit formation. The mechanism of biofilm inhibitors differs from that of biodispersants and enzymes in that bacterial attachment and biofilm formation is prevented by hindering the formation of a concentrated extracellular EPS layer around a bacterial cell. Bacteria lacking EPS have less capability to permanently attach to surfaces, cannot easily protect themselves from the harmful effects of microbicides, and do not provide “glue” to hold wet-end deposits together. Thus, in the idealized model of biofilm formation, sulfosuccinate works in stage 2, actually preventing the bacteria from forming an intact EPS matrix. The cells are then only loosely associated with the surface. Because of the absence of the concentrated EPS layer around the bacterial cell, sulfosuccinates are described as biofilm inhibitors to distinguish them from microbicides, biodispersants, and enzyme.

The first generation of biofilm inhibitors were studied in a European alkaline fine paper mill, a Scandinavian board mill, a European tissue mill, a North American tissue mill, and two North American alkaline fine paper mills (Bunnage et al., 2000). Before running any of the field trials, lithium tracer studies were conducted from which the system cycle-up was determined and the product application rates were accordingly determined. The control data from a European trial location reflect deposition on a machine with a conventional proprietary microbicide (dodecylguanidine hydrochloride and methylene bis (thiocyanate) and biodispersant in the short loop whitewater and a conventional proprietary microbicide (Bronopol and quaternary ammonium chloride) in the save-all. The control consisted of the paper machine running with a commercial biocide program. The treated system received a program consisting of the biofilm inhibitor. This conventional program was found to be effective at keeping the machine clean and providing the mill with a desired 4-week interval between boilouts. The microbicide and biodispersant feeding the short loop were completely replaced with a biofilm inhibitor program on an equal cost per ton basis. A side-stream monitoring device was used to measure visual appearance of the biofilm and the growth of certain biofilm constituents over a 2-week period. Both measurements showed a significant decrease in biofilm growth with the biofilm inhibitor program. Over an 11-month period, testing on this fine paper machine trial indicated that the biofilm inhibitor program reduced the number of breaks by 40%.

This provided the mill with a 103% return on investment. Also, the mill totally removed microbicides from the short loop of the machine, thus providing a much safer program. Results showed that, for the specific paper machine, continuous feed is preferential over semicontinuous. The trial consisted of various production runs with boilouts in between runs.

Olofsson et al. (2003) reported that *N*-acetyl-L-cysteine (NAC) may be an interesting candidate for use as an agent to reduce and prevent biofilm formation on stainless steel surfaces in environments typical of paper mill plants. Using 10 different bacterial strains isolated from a paper mill, the researchers found that the mode of action of NAC is chemical as well as biological in the case of bacterial adhesion to stainless steel surfaces. The initial adhesion of bacteria is dependent on the wettability of the substratum. NAC was shown to bind to stainless steel, increasing the wettability of the surface. Moreover, NAC decreased bacterial adhesion and even detached bacteria that were adhering to stainless steel surfaces. Growth of various bacteria, as monocultures or in a multispecies community, was inhibited at different concentrations of NAC. No detectable degradation of extracellular polymeric substances (EPS) by NAC was found, showing that NAC reduced the production of EPS, in most bacteria tested, even at concentrations at which growth was not affected. Altogether, the presence of NAC changes the texture of the biofilm formed and makes NAC an interesting candidate for use as a general inhibitor of formation of bacterial biofilms on stainless steel surfaces.

http://www.ncbi.nlm.nih.gov/pubmed?term=Olofsson%20AC%5BAuthor%5D&cauthor=true&cauthor_uid=12902275 US Patent 20,060,018,945 A1 (Britigan and Singh, 2006) provides a gallium-containing composition for coating/impregnating a device or device surface to prevent biofilm growth formation. The present invention also provides a method of preventing or inhibiting biofilm growth formation and for killing established biofilms.

Biosurfactants produced from the microbes were also found to impair biofilm-forming abilities. Biosurfactants produced by *Lactococcus lactis*–impaired biofilm formation on silicone rubber (Rodrigues et al., 2004). The ability of biosurfactant obtained from the probiotic bacterium *L. lactis* 53 to inhibit adhesion of four bacterial and two yeast strains isolated from explanted voice prostheses to silicone rubber with and without an adsorbed biosurfactant layer was investigated in a parallel-plate flow chamber. The microbial cell surfaces and the silicone rubber with and without an adsorbed biosurfactant layer were characterized using contact-angle measurements. Water contact angles indicated that the silicone-rubber surface with adsorbed biosurfactant was more hydrophilic (48°) than bare silicone rubber (109°). The results showed that the biosurfactant was effective in decreasing the initial deposition rates of *Staphylococcus epidermidis* GB 9/6 from 2100 to 220 microorganisms cm⁽⁻²⁾ s⁽⁻¹⁾, *Streptococcus salivarius* GB 24/9 from 1560 to 137 microorganisms cm⁽⁻²⁾ s⁽⁻¹⁾, and *S. aureus* GB 2/1 from 1255 to 135 microorganisms cm⁽⁻²⁾ s⁽⁻¹⁾, allowing for a 90% reduction of the deposition rates. The deposition rates of *Rothia dentocariosa* GBJ 52/2B, *C. albicans* GBJ 13/4A, and *Candida tropicalis* GB 9/9 were far less reduced in the presence of the biosurfactant compared with the other strains.

Surfactin from *B. subtilis* was found to disrupt biofilm without affecting cell growth and prevent biofilm formation of *Salmonella* enteric and *E. coli* (Mireles et al., 2001). Microbial molecules such as nisin, reuterin, and pediocin have been reported on their abilities to control biofilm formation by *L. monocytogenes* (Dufour et al., 2004). However, the use of biological control is not a cost-effective method in comparison to the chemical used. Because chemical disinfectants have been widely used to eliminate biofilms, the properties of the chemical have been concerned based on effectiveness, safety, easy applicability, easy rinsing off from the surfaces, no toxic residues are left that can affect the health properties and sensory values of the final products. In the past, efficiencies of biological and chemical disinfectants were previously tested on planktonic (free cell) rather than biofilm mode of growth. Biofilms have been reported to be 100–1000 times resistant to disinfectants (Gilbert et al., 2002). Thus, to identify the efficiency of disinfectant in the elimination of biofilm must be evaluated in the biofilm mode of growth.

A conventional microbicide strategy is still recommended for incoming furnish to maintain a consistent microbiological population level and as treatment for mill fresh water.

8.9 Bacteriophage Use

Phages are ubiquitous in nature. Bacteriophages are viruses that infect bacteria and may provide a natural, highly specific, nontoxic, feasible approach for controlling several microorganisms involved in biofilm formation (Kudva et al., 1999; Duckworth, 1976). Bacterial viruses or bacteriophages (phages) are hypothesized to be the predominant lifeform in the biosphere, clearly outnumbering their host bacterium. Bacterial viruses were discovered about 100 years ago (d'Hérelle, 1922; Hauduroy, 1925) and their discovery is attributed to Félix d'Hérelle, a French-Canadian microbiologist and Frederick Twort, an English bacteriologist (Twort, 1915; d'Hérelle, 1917). d'Hérelle named these viruses as “bacteriophages” from the words “bacteria” and “phagein,” which in Greek means to eat or devour. d'Hérelle performed extensive trials in humans and animals (Barrow and Soothill, 1997) and tried to shed a light on their nature and ability to function as therapeutic agents (d'Hérelle, 1922, 1917). He isolated bacteriophages for a number of bacterial hosts causing diseases. The phage products were produced in large scale at d'Hérelle's laboratory in Paris (today known as L'Oréal), and by several pharmaceutical companies (Eli Lilly & Co., Parke-Davis, Squibb & Sons, and Swan-Myers division of Abbott Laboratories, etc.) (Straub and Applebaum, 1993; Sulakvelidze et al., 2001; Summers, 1999). Twenty years after the official finding of phages, the first antibiotic, penicillin, was discovered. This fact, allied with some early clinical failures (Eaton and Bayne-Jones, 1934) and theoretical concerns did not continue with the phage therapy in the United States and most of the Western Europe. However, research and therapeutic use of phages continued in the Eastern European countries and the former Soviet Union (Alisky et al., 1998; Chanishvili et al., 2002; Slopek et al., 1987; Weber-Dabrowska

et al., 2000). In these countries, phages continued to be regarded as a good treatment method against a wide range of bacterial infectious diseases (Sulakvelidze et al., 2001; Ho, 2001).

Phages are currently suggested as possible alternatives to antibiotics for the treatment of bacterial diseases in humans and animals and widely explored to minimize the pathogen loads in food products of animal and plant origin. These phages do have a variety of advantages over chemical agents:

- Isolation is fast and simple
- Production is inexpensive
- Specific against a host or host range and thus do not affect the normal microflora of the environment where they can be applied
- Environmentally friendly
- Self-replicate at the infection site as long as the host bacterium is present
- No serious side effects reported

Not much information is available on the action of bacteriophages on biofilms (Hughes et al., 1998a,b; Sillankorva et al., 2004; Sutherland et al., 2004). The infection of biofilm cells by phages is extremely conditioned by their chemical composition and the environmental factors, such as temperature, growth stage, media, and phage concentration (Chaignon et al., 2007; Sillankorva et al., 2004). The major advantage of using phages in combating bacteria is their bactericidal (killing) nature, selectivity, and nontoxicity to man and the environment. The main drawback is that the phages have to be isolated for each harmful bacterium, the type of which may vary between paper mills. When phages make contact with biofilms, further interactions take place, depending on the susceptibility of the biofilm cells to the phage and to the accessibility of the receptor sites. The integrity of the biofilm may be quickly destroyed if the phage also contains polysaccharide-degrading enzymes or if significant cell lysis is affected by the phage. These microbes are noted for their high activity in the presence of host cells and selective lytic action. But, the industrial application of this technique has been slow. Viruses lack an independent metabolism and multiply only inside the living cells, using the metabolic machinery of the host cells. A bacteriophage can propagate only by coming into contact with its specific host bacterium. When this happens, the phage lyses the bacterium completely. Some of the bacteriophages that attack *E. coli* (ψ , λ , and T-4 phage) are very well known for their extensive contribution to development in molecular biology. The bacteriophage contains an icosahedral head of 50–100 nm in diameter, a long tail of about 100 nm in length with or without a sheath around it and six tail fibers at the end of its tail. The bacteriophage contains only protein with nucleic acid as gene. The bacteriophage selectively attacks its particular host bacterium. The gene of the bacteriophage is placed into the host bacterium, where it multiplies. The host cells also begin to produce the specific protein which constitutes the bacteriophage. A large number of bacteriophages are formed in the host cell, when the phages are released from the host, the cycle begins once again. The bacteriophage formed in

the host cell has the same properties and morphology as the original. The reproduced phages thus can constantly lyse the same host bacteria. The number of bacteriophages formed after one lytic cycle is called “burst size” and the time required for the cycle is called latent period. These values are reported for evaluating the activity of bacteriophage.

Bacteriophages are found to induce a wide range of polysaccharide-degrading enzymes in their hosts. Dispersion by induction of a prophage, followed by cell death and subsequent cell cluster disaggregation has been observed by Webb et al. (2003). However, phage enzymes are very specific and rarely act on more than a few closely related polysaccharide structures. Phages and bacteria can coexist symbiotically within biofilms, suggesting that they would make poor tools for the control of biofilm formation. Combinations of phage enzymes and disinfectants have been recommended as possible control strategies under certain conditions (Tait et al., 2002) with the phage added before addition of disinfectant being more effective than either of these alone. Mixtures of enzymes are commonly used, composed on arbitrary base.

Japanese researchers conducted investigations using *Pseudomonas* sp. (S-1) and its corresponding bacteriophage (pS-1) (Araki and Hosomi, 1990). The growth of slime-forming bacteria was inhibited in the presence of its corresponding bacteriophage. Results with bacteriophage and conventional biocides showed that after addition of 10 ppm of MBT to the test solution, the colony count of the slime-forming bacteria increased from 87×10^5 cfu/mL to 110×10^5 cfu/mL after 24 h at 28 °C showing that MBT was effective in keeping the activity of the slime-forming bacteria to a low level. However, the simultaneous addition of MBT and bacteriophage PS-1 reduced the colony count of the slime-forming bacteria from 100×10^5 cfu/mL to 2.4×10^5 cfu/mL. Results showed that addition of bacteriophage to mill whitewater was an effective technique for slime control and addition of bacteriophage with conventional biocides was also effective. Practical application of this technique on a commercial scale awaits completion of fundamental studies in several key areas. Bacteriophages will not impair the activity of the sludge used in waste treatment systems unlike conventional biocides.

Vaatanen and Harjy-Jeanty in 1986 reported the use of bacteriophages in paper mill whitewater systems. Their concept was based on the idea of isolating harmful bacterial streams of process waters and thereafter searching for virulent, lytic bacteriophage for these bacteria. They studied bacteriophages lytic for the bacteria *Enterobacter* and *Klebsiella* in model systems and found that phage activity against *Enterobacter agglomerans* stopped its growth for more than 19 h. When *K. pneumoniae* was dosed with phage at 3-h intervals, bacterial growth was no more restricted than when the culture was injected only at the beginning of the test. It was concluded that further studies to determine the efficacy of bacterial control in process waters should take into consideration the expected development of bacterial resistance to phage attack and the variation in bacterial strains among paper mills.

Sharma et al. (2005) observed the synergistic effect of an alkaline cleaner and a bacteriophage in the inactivation of *E. coli* O157:H7 biofilms formed on stainless steel. Lu and Collins (2007) engineered a bacteriophage to express a biofilm degrading enzyme. This enzymatic phage was found to have the ability to attack the bacterial cells in the biofilm and the biofilm matrix, considerably reducing the biofilm cell counts; more than 99.9% of removal was achieved. Hughes et al. (1998a) working on the control of *E. agglomerans* biofilms by the use of phages observed that the cells were killed and the biofilms were degraded by the bacteriophage. The phage then lysed the biofilm cells and the polysaccharide polymerase enzyme degraded the EPS and caused biofilm to slough off. Sillankorva et al. (2004) used bacteriophages to remove *P. fluorescens* cells, showing that phages were effective in the removal of biofilms in the early stage of development and 5-day-old biofilms under optimal conditions; up to 80% biofilm removal was obtained. Hanlon et al. (2001) have reported that in *P. aeruginosa* biofilms the bacteriophage migration through the biofilms is facilitated by the reduction in alginate viscosity. This phenomenon is related to the exopolysaccharide degradation by enzymes produced by the bacterial host. Hibma et al. (1997) reported that a bacteriophage (*L. monocytogenes* phage ATCC 23074-B1) was used successfully in *L. monocytogenes* biofilm inactivation. *E. coli* biofilms have been shown to be susceptible to bacteriophage T4 (Doolittle et al., 1995).

8.10 Electrochemically Activated Biocides

Electrochemically produced biocides can be a green alternative for organic biocides in use today (Ullah, 2011; Ashraf et al., 2014). These biocides are produced by electrolysis of diluted salt solutions in an electrolysis cell (Kiuru et al., 2010). The oxidation-reduction potential was found to be an important factor that defines the biocidal activity of electrolyzed anode water. These electrochemically generated biocides are environmentally friendly, nontoxic, hypoallergenic, chemical residue-free, fast-acting powerful biocide agents that do not require special handling and can be safely disposed of in municipal sewage systems (Robinson et al., 2010). Inactivation of microbes by electrochemical oxidation may happen in following two different ways:

- Direct anodic oxidation: Microorganisms are destroyed at the electrodes surface by hydroxyl radicals produced from water by electrolysis. Hydroxyl radical is the most powerful oxidant and it can cause oxidative stress against cell of bacteria.
- Indirect electrochemical oxidation: Microorganisms are destroyed by electrochemically produced ozone or hydrogen peroxide.

In paper and board machines, broke towers are usually the places where anaerobic conditions develop. Anaerobic bacteria are more sensitive to oxidizing compounds than aerobic bacteria and therefore electrochemically produced biocides are found to be powerful against anaerobic

bacteria. Bacterial spores are highly resistant to many conventional organic biocides. Oxidizing biocides such as hypochlorite are found to be more effective against spores. Electrochemically produced hypochlorite had a bleaching effect and that was evident as an ISO brightness increase of approximately 1 unit.

Electrochemical oxidation has been applied successfully to degrade different organic pollutants, such as phenols or industrial wastewaters, textile wastewaters, and olive mill wastewaters (Särkkä et al., 2008). Several terms are being used to represent solutions that are produced by the electrolysis of salts from passing an electric current (Ashraf et al., 2014). They are called

- Electrochemically activated solutions (ECAS)
- Electrolyzed oxidizing water
- Mixed oxidants (miox)
- Electrochemically activated water

The main molecule produced by electrolysis of sodium chloride solution of a given strength with biocidal potential is HOCl. It is an unstable hydroxy radical. The redox potential of these solution is between +800 and +1200 mV; pH ranges from 2 to 5 (Cloete et al., 2009; Thorn et al., 2012). Electrochemically produced biocides can be obtained using other salts, such as potassium chloride and magnesium chloride. These solutions have been extensively used in many industries. The primary oxidant is chlorine, produced from electrolysis of the salts in the aqueous media (Thorn et al., 2012; Buck et al., 2002). The next candidate used to make ECAS in the halogen series is bromine. It is an effective biological control agent. It rapidly degrades to harmless bromide. Therefore, it is not a persistent pollutant. Bromine is produced by electrolysis of a nonhazardous aqueous solution of NaBr and chloride. The electrolytic bromine can completely replace hazardous/toxic chemical biocides for the control of microorganisms in cooling towers (Timothy, 2007). Anaerobic bacteria are more sensitive to oxidizing compounds in comparison to aerobic bacteria, and therefore electrochemically generated biocides are powerful antianaerobes. Bacterial spores are highly resistant to many traditional organic biocides, whereas these ECAS have been effective against a number of pathogens, including *Bacillus atrophaeus*, *B. cereus*, *Bacillus anthracis*, human norovirus, hepatitis B virus, HIV, *Aspergillus flavus*, *C. albicans*, *S. aureus*, *P. aeruginosa*, *Enterococcus faecalis*, *Clostridium difficile*, *Cryptosporidium parvum* oocysts, and staphylococcal enterotoxin (Venczel et al., 1997; Shetty et al., 1999; Kim et al., 2000; Rogers et al., 2006; Park et al., 2007; Tagawa et al., 2000; Xiong et al., 2010; Morita et al., 2000; Zeng et al., 2011; Buck et al., 2002; Holcomb et al., 2005; Robinson et al., 2010; Raad and Sherertz, 2001).

In a study by Gareth et al. (2013), acidic ECAS were stored at temperatures of 4 and 20 °C for 398 days to examine the changes in the free chloride content, pH,

oxidation-reduction potential, and antimicrobial activity. The results showed a faster degradation at the higher temperature than at the lower temperature, with a concomitant decline in bactericidal capacity, whereas the pH did not change. The use of high concentrations of biocides can be avoided by adding greener biocide enhancers, such as ethylenediamine disuccinate (EDDS); its usage in the laboratory allowed the application of less glutaraldehyde to the test biofilms formed by sulfate-reducing bacteria. EDDS, which is a biodegradable chelator, increases the effectiveness of glutaraldehyde in its treatment of sulfate-reducing bacteria biofilms. This concept was initially used by Raad and Sherertz (2001) to treat biofilms on catheters (Raad et al., 2003, 2007) but with the new concept of green materials, ethylenediaminetetraacetic acid (EDTA) was a noncompatible molecule because of its slow biodegradability, and it was replaced with environmentally compatible chelating agents. The activity of biocides such as THPS can be increased by the use of EDDS.

Eversdijk et al. (2012) designed a biocide controlled-release system that consisted of modified nanoclay particles incorporated with different model biocides, and studied their release and antifungal activities under different environmental conditions. This composite strategy allowed the production of a tunable system to control the release rate and extend its effectiveness to 45 days during an artificially generated rain test. This approach appears promising for the prolonged protection of different construction materials and waterborne paints. Routine coatings last for an average of half a year or a maximum of 2 years, whereas the desired service life in buildings is at least 10 years. The effect of a controlled-release system on biocide concentrations over time was studied. Increasing the dose of a biocide was found to result in a minor prolongation of protection, but increased in-house environmental pollution. To meet the challenges to material performance and environmental safety, such durable composite materials are a beam of hope for safer green applications (BPD, Directive 98/8/EC, 2012).

Eguia and Trueba (2007) applied marine biotechnology to produce environmentally benign water-based coatings using lower-toxicity elements and bacteria isolated from surfaces immersed in marine water. These promising coatings were able to reduce the problem of biofouling while having no adverse effects on the surrounding environment.

Electrochemically generated biocides showed an effective way to control microbial problems at a paper mill (Ullah, 2011). They can be added to water or pulp and they have hardly any negative effect on the process or end-product. For paper makers, this study is of great interest because onsite-generated biocides are low cost solutions based on actual biocide need. Onsite-generated biocides also eliminate the storage and transportation of biocides and provide a basis for building a new control program. These electrochemically generated biocides are fast-acting, powerful biocide agents, used during all stages of disinfection and

cleaning, applied in liquid, aerosol or frozen forms, chemical residue-free, generated onsite or in concentrated amounts for imminent use, eliminating handling and storage issues, and produced from municipal tap water and salt.

Flores et al. (2013) studied the ability of citrate-capped silver nanoparticles (AgNPs) to stop the formation of bacterial biofilms by *S. aureus* and *P. aeruginosa*. These are clinically important gram-positive and gram-negative bacteria. They used two different approaches. The first approach involved the dispersal of AgNPs to study the bactericidal effect on planktonic bacteria, whereas the second approach was to adsorb AgNPs on a titanium substrate to test their bactericidal effect on sessile bacteria. Planktonic *P. aeruginosa* was found to be more susceptible to nanoparticles than was *S. aureus*, which can be attributed to their different cell wall structures. Similar results were obtained for the sessile species. The results also revealed that performance was improved by using titanium-based implants, even at very low concentrations.

Hussein et al. (2013) produced chitosan (a biopolymer), by extracting the exoskeletons of crustaceans found in seafood waste. It was 85.2% deacetylated and had an average molecular weight of 109 kDa. Chitosan was derivatized, forming 2-N,N-diethylbenzene ammonium chloride N-oxoethyl chitosan, called compound I, and 12-ammonium chloride N-oxododecan chitosan, ecofriendly inhibitors of carbon steel corrosion in low-pH media. The results demonstrated that compound I was the more efficient corrosion inhibitor. Comparing the antibacterial activity of chitosan and its derivatives indicated that chitosan (compound II) was more effective than its derivatives against several bacteria: *C. albicans*, *E. coli*, *E. faecalis*, and *S. aureus*.

Yuan et al. (2012) attempted to reduce biocorrosion by sulfate-reducing bacteria. This approach involved a combination of surface-initiated atom transfer radical polymerization and in situ chemical oxidative graft polymerization techniques. Stainless steel was tethered with a poly(4-vinylaniline)–polyaniline (PVAn-PANI) bilayer layer coating. This method involved three steps: in the first step, a trichlorosilane coupling agent was immobilized on the stainless steel surface, providing sulfonyl halide groups for the ATRP of PVAn; in the second step, PANI was grafted onto the PVAn in situ; in the third step, the PVAn-PANI bilayer reduced bacterial adhesion and biofilm formation on the stainless steel surface, as showed by electrochemical test results, confirming that their anticorrosion and antibacterial properties were suitable for severe environments.

Hakkinen et al. (2004) has reported electrochemical microbial antifouling technology for the prevention of biofilm and inorganic deposits on stainless steel surfaces. This is based on polarization of the steel surface. A preventive current is passed from an electrode to the (metal) surface to be protected through an electrolyte, leading to oxidation and reduction reactions. These reactions temporarily change the pH of the metal surface. This creates

conditions that prevent microbial attachment. Several UPM mills are running full-scale microbial antifouling technology systems successfully.

8.11 Other Techniques in Biofilm Treatment

Research work for exploring possibilities of photocatalytic TiO₂ coating for reducing biofilms on nonliving surfaces has been investigated by Raulio et al. (2006). The model organism, *Deinococcus geothermalis*, known to initiate growth of durable, colored biofilms on machine surfaces in the paper industry, was allowed to form biofilms on stainless steel, glass and TiO₂ film-coated glass or titanium. When biofilms on photocatalytic TiO₂ surfaces, submerged in water, were exposed to 20 W h m⁻² of 360 nm light, both kinds of adhesion threads were completely destroyed and the *D. geothermalis* cells were extensively removed (from >10⁷ down to below 10⁶ cells cm⁻²). TiO₂ films prepared by the sol-gel technique were slightly more effective than those prepared by the ALD technique. Doping of the TiO₂ with sulfur did not enhance its biofilm-destroying capacity. The results show that photocatalytic TiO₂ surfaces have potential as a self-cleaning technology for warm water using industries.

Also antifouling potential of electric polarization combined and not combined with biocides was studied in nonsaline warm water with high organic content by Peltola et al. (2011). When *D. geothermalis* biofilms grown for 24 h in simulated paper machine water were exposed to cathodic or cathodically weighted pulsed polarization at least 60% ($P < 0.05$) of the biofilms were removed from stainless steel (AISI 316L). Biofilm removal by 25 ppm (effective substances 5–25 ppm) of oxidizing biocides (bromochloro-5,5-dimethylhydantoin, 2,2-dibromo-2-cyanoacetamide, peracetic acid) increased to 70% when combined with cathodically weighted pulsed polarization. Using a novel instrument that allows real-time detection of reactive oxygen species, these researchers showed that the polarization program is effective in antifouling generated reactive oxygen species in a pulsed manner on the steel surface. They suggested that the observed added value of oxidative biocides combined with polarization depended on reactive oxygen species. This suggestion was supported by the finding that a reductive biocide, methylene bithiocyanate, counteracted the antifouling effect of polarization (Table 8.39).

Table 8.39: Comparison of different methods used for biofilm prevention at paper mills

Methods	Effectiveness	Ease of use at mill	Cost/ton of paper
Physical cleaning	High	Challenging and slow	1–2 euros
Biocide treatment	High	Easy and fast	1–4 euros
Biodispersants	Low	Easy and slow	1–4 euros
Electrochemical treatment	High	Challenging and slow	5–10 euros

Based on https://noppa.lut.fi/noppa/opintojakso/.../biocides_case_study.pdf.

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Future Prospects

Deeper scientific insight is required that will enable the development of more effective biocontrol strategies in the paper process that will improve the efficiency of the process and the quality of the product. Successful strategies against biofouling should be based on integrated approaches (Table 9.1) that consider the entire system to be protected (Flemming, 2011). Conventional methods do not address the primary causes of the biofouling problem. The most important components of conventional methods are those that destabilize the extracellular polymeric substances matrix by using cleaning agents. The usual practice is to apply a mixture of biocides, inhibitors, and dispersants, mainly based on experience. The most promising approaches include good housekeeping to minimize the fouling potential of the water phase by keeping the bacterial numbers and nutrients as low as possible (Blanco et al., 1996). Easy-to-clean surfaces and materials that do not support microbial adhesion and growth are another element of integrated antifouling strategies (Whitehead and Verran, 2009; Vladkova, 2009; Roukolainen et al., 2008; Schackenraad et al., 1992; Marmur, 2004; Genzer and Efimenko, 2006; Bers and Wahl, 2004; Carman et al., 2006; Zhao et al., 2002; Sunada et al., 2003; Perez-Roa et al., 2006; Schaule et al., 2008; Giladi et al., 2008; Louie et al., 2006; Marshall and Blainey, 1990; Wood et al., 1996; Huttinger et al., 1982; Tiller et al., 2002; Milovic et al., 2005; Madkour et al., 2008; Zasloff, 2002; Leeming et al., 2002; Parvici et al., 2007; Lewis and Klibanov, 2005; Klibanov, 2007; Majumdar et al., 2008). Establishment of an early warning capacity is very important to initiate timely countermeasures. Surface monitoring will help a lot, either performed by regular sampling of accessible surfaces or by following fouling-related parameters. An integrated approach appears more realistic and sustainable (Flemming et al., 2013; Flemming, 2003; Jahnknecht and Melo, 2003; Hillman et al., 1985; Strathmann et al., 2013; Klahre and Flemming, 2000; Rice and Schild, 2009; Macuch and Rice, 2008). These approaches are expensive and complex,

Table 9.1: Major elements of an integrated antifouling strategy

Low bacterial numbers in the water phase
Adhesion-repellent surface, polarization, etc.
Limitation of biofilm growth by limitation of nutrients
Cleaning-friendly design; easy-to-clean surfaces
Surface monitoring:
• Early warning
• Cleaning control

Based on Fleming (2011).

but may be cost-effective in the long term and will be more sustainable in comparison to present strategies.

The application of enzymes to control biofilm in the paper industry is not very common. The main emphasis of research has been on characterizing the different types of extracellular polymeric substances produced by microorganisms to develop methods for removing biofilms. Using enzymes to remove bacterial biofilm is limited due to the low prices of conventional chemicals used in the industry; another reason is the lack of reliable methods for the quantitative evaluation of the enzymatic effects. Also the commercial accessibility to some enzyme formulations is restricted. It is known that monocomponent enzymes can be used for biofilm removal, but the diversity of the biofilm matrix limits the potential of specific enzymes. So, it is necessary to use a wide range of enzyme activities to attain an acceptable degree of removal in complex biofilms (Torres et al., 2011, 2012).

Future studies should focus on the mechanism of the deposit formation at paper machines in combination with developing deposit control agents to meet the industrial demands of efficiency as well as environmental sustainability (Kanto Öqvist, 2008). This might also include new treatment (e.g., coating of stainless steel with diamond-like carbon and fluoropolymers) methods of the steel to prevent or influence the attachment mechanism of the microbes and other substances (Raulio et al., 2008; Kallio and Kekkonen, 2005). The understanding and implementation of such studies would lead to less toxic antifouling products as well as individually designed more cost-efficient programs for each paper machine in the future.

Biocide research today should consider the toxicological and environmental impacts. Therefore, research is focused on making biocides more effective at lower concentrations. Efforts are also being made to develop nontoxic and environmentally friendly products. Furthermore, many agents already known for years to be effective as biocides or as disinfectant chemicals but considered to be costly have been put into reuse. Examples are hydrogen peroxide, glutaraldehyde, ozone, and peracetic acid. For controlling the microbiological population, sometimes a full count of microbial species at different locations is required, rather than determining the presence of certain microbial groups like slime-forming species, yeasts, anaerobic bacteria, and fungi, etc. Traditional methods for identifying microbes include enumeration and screening of individual species isolated from count plates on selective media. Ready-made plates are available for the selection of different types of microorganisms. Commercial identification systems based on several biochemical tests or other properties are available, including a database that covers virtually all different types of bacteria.

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